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ABSTRACT BOOK

Sunday, October 2, 2022

Oral Presentations

22IR11: Biological Applications of Vibrational Spectroscopy

Chair: Mike George

Co-Chair: Curtis Marcott

(IR-11.1) Breaking the Thiol Barrier: N-Heterocyclic Carbenes as a Robust Platform for Bioconjugation and Sensing

Jon P. Camden¹; ¹*University of Notre Dame*

Bioconjugation using N-heterocyclic carbenes (NHCs) is more robust than the thiol based methods.

To date, surface functionalization of noble metal surfaces relies almost exclusively on thiol-based self-assembled monolayers (SAMs). However, thiols suffer from a plethora of problems: in addition to the challenging synthesis of thiol precursors, the resulting monolayers are not stable in aerobic or acidic conditions or under electrochemical potentials. N-heterocyclic carbenes (NHCs) provide an exciting alternative to thiols as they are known to yield monolayers with superior stability and further provide a platform from which post-synthetic modifications can be carried out. In this talk, I will discuss the fundamental surface chemistry and spectroscopy of NHC functionalized surfaces as well as emerging applications in electrochemical sensors.

(IR-11.2) Spectroscopic Studies Related to the Etiology of Dry Eye and Cataract

Douglas Borchman¹; ¹*University of Louisville*

Infrared and NMR spectroscopies were used to elucidate the etiology of cataracts and dry eye

NMR and Infrared spectroscopies were instrumental in determining the relationships between lens and tear lipid composition, conformation and function. The major lipid of the human lens is dihydrosphingomyelin, discovered by NMR spectroscopy and found in quantity only in the lens. The lens contains a cholesterol to phospholipid molar ratio as high as 10:1. Lens lipids contribute to maintaining lens clarity, and alterations in lens lipid composition due to age are likely to contribute to cataract. Lens lipid composition reflects adaptations to the unique characteristics of the lens: no turnover of lens lipids or proteins and contains almost no intracellular organelles. Long-lived species such as humans and the bowhead whale exhibits lens lipid adaptations that confer resistance to oxidation, and thereby allowing the lens to stay clear for a relatively longer time than is the case in many other species. With cataract, light scattering increases due to the increase in the lipid order of lens membranes measured using infrared spectroscopy. It is plausible that the increase in lipid-lipid interactions may contribute to myopia by causing greater compaction and overall stiffness of the lens. The tear film lipid layer (TFLL) is a thin, 100 nm layer of lipid on the surface of tears covering the cornea that contributes to tear film stability. NMR spectroscopy found that the major lipids of the TFLL are wax esters and cholesterol esters.

The hydrocarbon chains associated with the esters are longer than those found anywhere in the body, as long as 32 carbons, and many are branched. More ordered lipid with dry eye, measured using FTIR, could inhibit the flow of meibum from the meibomian glands and contribute to the formation of a discontinuous patchy TFL, which in turn results in deteriorated spreading, and decreased surface elasticity. One may also speculate that more ordered lipid results in the attenuated capability to restore tear film lipid layer structure between blinks.

(IR-11.3) Development and Evaluation of a Non-Contact Raman Spectroscopy Probe for In-Vivo Characterization of Otitis Media

Sean Fitzgerald¹, Guillermo Monroy², Alexander Ho², Andrea K. Locke¹, Stephen A. Boppart², Anita Mahadevan-Jansen¹; ¹*Vanderbilt University*, ²*University of Illinois at Urbana-Champaign*

This work presents a miniature Raman Spectroscopy probe design for non-contact acquisition of spectra in-vivo.

Raman Spectroscopy (RS) provides non-invasive and label-free quantification of tissue composition through inelastic scattering of light. Recent advancements in RS systems designed for in-vivo applications utilize hand-held fiber optic probes that offer steric freedom for collecting RS spectra from virtually any location within the body. While this form of optical spectroscopy has shown great potential to characterize superficial and even internal tissues in contact mode, it is challenging to use in applications where the probe cannot directly contact the target tissue. This is because coupling efficiency of diffusely reflected Raman emissions into a fiber optic degrades as the probe is separated from the tissue surface. Also, controlling the distance of probe tip relative to tissue surface is challenging without some form of real-time feedback. Here, a novel design for a small diameter non-contact RS probe is presented for application in Otitis Media (OM), an inflammatory disease of the middle ear. This probe uses a micro-lens to reimage the excitation/collection fiber end onto the tympanic membrane (TM), while remaining small enough to be inserted through a standard ear speculum for collecting spectra from the middle ear *in vivo*. This design demonstrates improved collection efficiency versus a standard lens-less RS probe design. To provide axial-positioning feedback, a range-sensing approach using Low Coherence Interferometry (LCI) is integrated into the probe to help control probe-to-tissue distance, allowing for repeatable RS scans in non-contact mode.

(IR-11.4) Surface-Enhanced Raman Spectroscopy of Bacterial Metabolites to Unveil Bacterial Tolerance to Antibiotics

Wei Wang¹, Peter J. Vikesland¹; ¹*Virginia Tech*

The SERS results suggest bacterial secondary metabolite pyocyanin modulates its antibiotic tolerance

The presence of antibiotics in waterbodies can facilitate the development and proliferation

of antimicrobial resistance (AMR) - one of the greatest public health threats currently facing humankind. An improved understanding of AMR is of great societal importance. In this study, we report the use of surface enhanced Raman spectroscopy (SERS) for the monitoring the bioactive metabolites of two ampicillin resistant *Pseudomonas aeruginosa* strains and the identification of bacterial antibiotic resistance mechanisms. The time-dependent SERS results show that multiple bioactive metabolites can be determined during bacterial growth and the blue-green pigment pyocyanin (PYO) dominates. PYO accumulates during the early growth stage and is subsequently consumed or diffuses into the culture medium. In the presence of ampicillin at concentrations below the minimal inhibitory concentration (MIC), *P. aeruginosa* growth is maintained at a rate consistent with the control. The nutrient consumption and the production of most of the metabolites are not affected. However, the SERS signal of PYO is strongly promoted. PYO acts as a quorum sensing signaling molecule and the increase of PYO concentration can promote the transcription of antibiotic resistance genes. We further detected the metabolic SERS signals of ampicillin susceptible *Escherichia coli* and found that exogenously added PYO promotes *E. coli* growth, even in the presence of ampicillin. The results indicate that PYO confers antibiotic tolerance not only for the producing species, but also other cocultured bacterial strains. Our work provides new techniques and insights to better understand bacterial antibiotic tolerance mechanisms and has implications for effectively addressing the threat of AMR.

(IR-11.5) Application of Infrared Spectroscopy to Study the Stability of Biological Samples

Anna Wójtowicz¹, Marcin Reciak¹, Renata Wietecha-Posłuszny¹; ¹*Jagiellonian University*
Changes in FTIR spectra of vitreous humor and liver stored under various conditions were identified.

The stability and degradation processes of the biological matrix are of great importance in forensic analyzes. Knowledge of their course can provide valuable information about the time and conditions of exposure of samples to external factors. Furthermore, it is also important to test the matrix stability during sample storage prior to analysis, which is most often done by freezing (-20 ° C) or cooling (4 ° C). In this type of research, it can be advantageous to use vibrational spectroscopy methods, such as attenuated total reflectance Fourier transform infrared spectroscopy (ATR FTIR), which is a fast, simple, and non-destructive technique, with literature examples of an effective application for the analysis of postmortem samples [1].

The purpose of the study was to investigate the stability of two alternative biological matrices: vitreous humor and liver homogenate during 30 days of storage at three temperatures of -20, 4, and 20 ° C. The research was carried out on postmortem bovine samples of vitreous humor and liver. The samples were deposited on microscope slides and after 24 hours of drying at room temperature, they were scraped with a scalpel and measured on a diamond crystal FTIR spectrometer. The principal component analysis (PCA) method was used for statistical data analysis.

Significant biochemical changes were observed mainly in the structure of proteins, polysaccharides, fatty acids, and amino acids, and they occurred most quickly at 20 ° C, then 4 ° C, while storage at -20 ° C ensured stability of both tested matrices. The observed spectral changes, their kinetics, and a comparison of the results obtained for both tested matrices will be discussed.

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[1] Wójtowicz, A., Mitura, A., Wietecha-Posłuszny, R. et al. Spectroscopy as a useful tool for the identification of changes with time in post-mortem vitreous humor for forensic toxicology purposes. *Monatsh Chem* 152, 745–755 (2021).

22RAM14: Higher Order and Advanced Techniques

(RAM-14.1) Determination of Second Hyperpolarizability with Computational Raman Activities and Identification of DOVE Signatures for Selected Molecules

Wei Zhao¹; ¹*University of Arkansas at Little Rock*

Predetermination of DOVE four wave mixing signatures using computationally determined second hyperpolarizability

Doubly vibrationally enhanced (DOVE) four wave mixing spectroscopy, as an optical analogue to 2D NMR, involves two infrared transitions and a Raman transition. The magnitude of the DOVE second hyperpolarizability γ can be theoretically estimated if the values of the dipolar moments of the two infrared transitions and the γ of the Raman transition are known. The Raman γ can be measured by using four wave mixing interferometric method or conventional Raman spectroscopy in the presence of an internal standard. Here, we have demonstrated that the second hyperpolarizability γ of a selected vibrational mode of a molecule can be determined by using the computational Raman activity against an internal standard with a known Raman γ value. This approach provides a convenient way for prediction of the γ magnitude of DOVE four wave mixing spectroscopy. By using the Hartree-Fock (HF) method, the DFT method, and the MP2 method, our work covers from the less anharmonic region $< 2000 \text{ cm}^{-1}$ to the more anharmonic region $> 2000 \text{ cm}^{-1}$ covering C-H, C-D and C \equiv N stretching modes of benzene, deuterated benzene, acetonitrile, deuterated acetonitrile, and tetrahydrofuran. By choosing a suitable method and basis set, this facile approach could be applied to a broader spectral range for Raman γ estimation of various materials. Further work is extended to estimate the magnitude of the DOVE second hyperpolarizability of some of the listed samples and identify the strong DOVE signatures for label-free molecular determination in a complex system.

(RAM-14.2) White Light Continuum Generation in Bulk Media Triggers High-Speed Multiplex CARS in the Fingerprint Region

Dario Polli¹, Federico Vernuccio¹, Arianna Bresci¹, Alejandro De La Cadena¹, Benedetta Talone¹, Chiara Ceconello¹, Francesco Manetti¹, Subir Das¹, Renzo Vanna², Giulio Cerullo¹; ¹*Politecnico di Milano*, ²*CNR-Institute for Photonics and Nanotechnologies (IFN-CNR)*

We demonstrate high-speed broadband CARS in the fingerprint region generating supercontinuum in a YAG crystal.

Coherent anti-Stokes Raman Scattering (CARS) microscopy is a very powerful imaging technique that determines the molecular composition of cells/tissues by recording their vibrational response in a label-free manner. Its simplest implementation employs two narrowband picosecond pulses (pump and Stokes) to probe a single vibrational mode. Broadband CARS (B-CARS) combines a narrowband pulse (the pump) with a broadband pulse (the Stokes) and aims at recording a full Raman spectrum in a single shot. Despite many improvements in the last decade, B-CARS microscopes struggle to work in the so-called “fingerprint” spectral region (400-1800 cm^{-1}), because it features weaker Raman response than the high-frequency CH-stretching region (2800-3100 cm^{-1}), even if it provides higher biochemical specificity. Furthermore, to generate the broadband Stokes, B-CARS systems typically employ photonic-crystal fibers (PCFs), which are sensitive to misalignments and add spectral noise, hindering the mainstream use of B-CARS.

In this work, we propose a novel approach to B-CARS to solve these issues. Our experimental setup employs a femtosecond laser with a lower repetition rate than standard systems ($\gg 2$ MHz vs. $\gg 80$ MHz), thus delivering pulses with much higher (\gg mJ level) energy. This unlocks the possibility to produce broadband Stokes pulses by white-light continuum generation in a bulk YAG crystal, which is a compact, robust and alignment-insensitive technique. Moreover, the reduced repetition rate allows illuminating the sample with higher pulse energy, thus generating stronger CARS signals. In this way, we demonstrate state-of-the-art acquisition speed (< 1 ms/pixel, limited by the spectrometer refresh rate) with unprecedented sensitivity ($\gg 14.1$ mmol/L), covering the whole fingerprint region. In parallel, we design a post-processing pipeline to identify different chemical constituents in heterogeneous biological samples, that includes an innovative spectral denoiser based on a convolutional neural network, followed by the Kramers-Kronig algorithm for the removal of the non-resonant background, generated by an out of resonance four-wave mixing process, and several numerical algorithms for chemical classification. Thanks to this approach, the B-CARS microscope can deliver high-quality and high-signal hyperspectral images at high speed, allowing us to identify the main Raman features for solvents, plastic microparticles and heterogeneous biological samples, such as tissue slices of murine spine.

(RAM-14.3) Probing Coupled Folding and Binding Processes of Ribonuclease S with Temperature-Jump Multidimensional Infrared Spectroscopy

Yumin Lee¹, Brennan Ashwood¹, Andrei Tokmakoff¹; ¹*University of Chicago*

Biomolecular spontaneous association and self-assemblies are essential to numerous cellular functions and processes of proteins such as metabolism, signaling, and gene regulation. To gain insights into the dynamic molecular events involved in recognition and association, we investigate a model noncovalent binding protein system, ribonuclease S (RNase S). Investigating the binding mechanism of RNase S consisting of two proteolytic fragments, S-protein and S-peptide, has made use of various biophysical experimental and computational approaches but questions remain regarding the molecular-level details of their interactions. We investigated the time-dependent properties of order-to-disorder conformational transitions coupled with dissociation of RNase S by employing laser-induced temperature-jump methods that use infrared pump-probe and 2D spectroscopic probes (T-jump PP/2DIR spectroscopy). It allows us to track non-equilibrium dynamics changes in protein secondary structure from nanosecond to multiple minute time delays. After initiating unfolding and dissociation of RNase S with a T-jump, we observed the transient infrared spectra associated with two different responses on tens of milliseconds and a few seconds time scales. Applying global lifetime analysis, we interpret how the spectral feature changes associated with each decay timescale correlate to the unfolding and complex deformation of RNase S. Aided with equilibrium 2DIR, FTIR, CD, and ITC measurements, we determine thermodynamic parameters and binding free energies correlated to the transition rates. We are extending this approach to the RNase S system with a single site-specific isotope ($^{13}\text{C}^{18}\text{O}$)-labeled S-peptide to decipher conformational change in a specific residue throughout the binding coupled folding process.

(RAM-14.4) **Identifying Biomolecular Changes in Murine Cortical Tissue After Blast-Induced Traumatic Brain Injury Using Coherent Anti-Stokes Raman Scattering Microscopy**

Jacob Hardenburger¹, Pratheepa Rasiah¹, Anita Mahadevan-Jansen¹; ¹*Vanderbilt University*

CARS microscopy is a novel technique to study trauma brain injuries *in vivo*.

Traumatic brain injury (TBI) is a leading cause of injury deaths in the US, responsible for more than 56,000 deaths every year, those who survive often deal with long-term side effects from the injury. The long-term effects of TBIs have been extensively studied, with most previous studies focusing on the effects of TBIs on timescales ranging from days to months to years. However, little research has been conducted to evaluate the biophysical changes induced by a TBI immediately after injury due to imaging techniques that lack molecular contrast. We aim to evaluate the biomolecular changes of *in vivo* murine cortical tissue directly after a blast-induced TBI using coherent anti-stokes Raman scattering (CARS) microscopy. This label-free imaging technique provides molecular contrast to detect biophysical changes in real-time. Craniotomies were performed on Cx3cr-1 transgenic mice anesthetized with urethane to expose the cortex before placing the mice under the non-linear imaging arm of the Multimodal Advanced Nonlinear and Thermal Imaging System (MANTIS). A hyperspectral focusing method was employed to collect spectral CARS images from 2700 cm^{-1} to 3200 cm^{-1} , using chirped femtosecond laser pulses with the stokes laser beam fixed at 1040 nm and the pump laser beam tuned to 798 nm. Spectral image stacks were acquired from the mice before and after being subjected to a 15-psi air blast. CARS data was extracted from the brightest scattering regions in the spectral stacks and analyzed in MATLAB. Post-blast CARS images show spectral shape

changes in the high wavenumber region of the Raman spectrum minutes after the blast injury with a significant increase in the ratio between 3020 cm^{-1} to 2930 cm^{-1} , Raman peaks attributed to biological lipids and biological proteins. This study shows CARS microscopy's ability to detect biomolecular changes in the cortex immediately after TBI exposure.

(RAM-14.5) Stable Isotope Raman Microspectroscopy: Applicability for Analysis of Microbial Degradation of Microplastics

Natalia P. Ivleva¹, Julian Weng¹, Kara Müller¹, Martin Elsner¹; ¹*Technical University of Munich (TUM)*

Stable isotope Raman microspectroscopy is a promising method for analysis of microbial degradation of microplastics

Stable isotope-based analytical methods are gaining increasing relevance and importance in different scientific fields. Although mass spectrometry-based methods enable sensitive analysis of bulk samples (e.g., isotope ratio mass spectrometry) or provide a spatial resolution down to 50 nm (e.g., nanoscale secondary ion mass spectrometry), these methods are destructive and require time-consuming sample preparation. Here, a combination of Raman microspectroscopy with the stable isotope approach – stable isotope Raman microspectroscopy (SIRM) – can extend the capabilities of the well-established techniques with a nondestructive, quantitative and spatially-resolved analysis. SIRM provides characteristic fingerprint spectra of samples with the spatial resolution of a confocal optical microscope, containing information on stable isotope-labeled substances and the amount of a label (based on red-shift of bands of the labeled substances). Simultaneously, these spectra deliver information on the chemical composition and structure of samples. Furthermore, this method requires no or limited sample preparation, and can be performed *in situ* without spectral interference of water. SIRM provides information on the carbon metabolism / flow and the cell activity, and hence can be suitable for the analysis of microbial degradation of the most prominent emerging pollutant in the (aquatic) environment – microplastics.

Here we present the feasibility study of SIRM for quantitative analysis of (micro)plastic biodegradation. As a model organism, we used carotenoid forming bacterium *Shingomonas koreensis*, isolated from suspensions of aged polylactide (PLA) microparticles. Both resonance Raman spectra of carotenoids and regular Raman spectra of biomass were acquired and compared in their applicability for ¹³C-isotope tracking, based on gradual red-shift of (resonance) Raman signals. While carotenoid signals showed considerable offsets for labeled bacteria, less pronounced changes were found for bands of biomass spectra, indicating that carbon flow can be monitored earlier in carotenoid spectra. Since stable isotope-labeled polymers are expensive or even unavailable, alternative approaches – reverse ¹³C-labeling SIRM and the use of D-labeled PLA are under evaluation. Overall, the labeling experiments show that SIRM enables for reliable analysis of stable isotope flows into microbial biomass at the single-cell level, and has a high potential for monitoring of the (micro)plastic biodegradation.

22SPECIAL12: Ordered Assemblies and Prepared Surfaces

(SPEC-12.1) Chiral-Specific Vibrational and Electronic Spectroscopy of Ordered Assemblies

Garth J. Simpson¹; ¹*Purdue University*

A mathematical framework is proposed for relating large chiral-specific spectroscopic measurements to structure and orientation.

The reduction in symmetry arising in ordered systems opens up new spectroscopic methods for probing chirality that are fully electric dipole-allowed and can be comparable in magnitude to their achiral counterparts. Molecular chirality is often a defining property of biologically active molecules, but often accessible optically through relatively weak effects, including coupling of magnetic and electric dipoles. However, these same molecular species can produce large chiral-specific observables in uniaxial assemblies. In this work, we demonstrate a relatively simple theoretical framework for providing molecular interpretations of previous reported chiral-specific fluorescence, absorbance, nonlinear optics, and surface-enhanced Raman spectroscopies, and predict new chiral-specific spectroscopic modalities.

(SPEC-12.2) Spectroscopic and Microscopic Tracking of Multicomponent Supramolecular Nanostructures with Optoelectronic and Energy Transfer Properties

Md Shah Alam¹, Jon Parquette¹, Karthikeyan Perumal¹, Jenae Linville¹; ¹*The Ohio State University*

Novel and highly complex nanostructures with optoelectronic and energy transfer properties.

Biological systems such as protein, DNA, and photosynthetic chlorophyll complexes are endless motivation to create synthetic complex multicomponent supramolecular nanostructures that closely mimic the complexity and multi-functionalities of the biological systems. Unlike conventional covalent polymers, supramolecular multicomponent copolymers are novel classes of self-assembled nano-systems that deliver exceptional photophysical properties relative to their constituent monomers. To achieve this aim, multiple chromophore molecules are often exploited in co-assembly for the construction of functional hierarchical nanostructures. The higher ordered nanostructures (i.e., lamellar nanofibers) are fabricated from the supramolecular self-assembly of constituent monomers comprised of four lysine motifs appended with 1,4,5,8-naphthalene diimide (NDI-lys) that is attached to a central 5, 10, 15, 20-tetra(4-aminophenyl) porphyrin (TAPP) derivative, a structural analog of chlorophyll as an integral part of the biological photosystems. The photophysical and morphological properties of supramolecular lamellar nanofibers are characterized by AFM, TEM, absorption, and circular dichroism in different experimental conditions. In addition, multicomponent nanostructures are developed by self-assembly of optically distinct derivatives of ethoxy and/or dithiol core-substituted naphthalene diimide di-lysine (cNDI-

Bola) where two lysine motifs are attached to the opposite ends of the NDI molecule. One critical aspect of such a hierarchical process is how the multiple self-assembling components interact to give well-defined multicomponent nanostructures with monomer sequence control. However, it is challenging to track the individual monomers spectroscopically and microscopically in their assembled states due to the dynamic exchange of components during the self-assembly which is essential for evaluating the functionalities of the resultant structures. Mechanistic studies on multicomponent nanostructures are investigated with the combination of various analytical tools such as fluorescence, circular dichroism, and UV-visible spectroscopy. Moreover, super-resolution structured illumination microscopy (SIM) was utilized to visualize the monomer sequence in complex hierarchical nanostructures with distinct emission profiles. Finally, the Förster resonance energy transfer (FRET) between the assembling components in the multicomponent nanostructures is evaluated. These novel classes of nanostructures have potential applications in optoelectronic and light-harvesting devices.

(SPEC-12.3) Aerosol Jet Printed SERS Substrates for Ultrasensitive Detection of PFAS

Rahul Rao¹, Colleen McDonnell¹, Faris Albarghouthi², Ryan Selhorst¹, Aaron Franklin²;

¹*Air Force Research Laboratory*, ²*Duke University*

Developed aerosol jet printed SERS substrates using Ag and graphene inks for ultrasensitive PFAS detection

Printing technologies offer an attractive means for producing low-cost surface-enhanced Raman spectroscopy (SERS) sensors with high throughput. The development of these sensors is especially important for field-deployable detection of environmental contaminants. Towards this end, we demonstrate surface-enhanced Raman spectroscopy-based sensors fabricated through aerosol jet printing of silver nanoparticle and graphene inks on Kapton films. Our printed arrays exhibited measurable intensities for fluorescein and rhodamine dyes down to concentrations of 10^{-7} M, with the highest SERS intensities obtained for four print passes of Ag nanoparticles. The sensors also exhibited an excellent shelf-life, with little reduction in fluorescein intensities after 9 months of shelf storage. We also demonstrated the capability of our sensors to detect perfluoroalkyl substances (PFAS), the so-called forever chemicals that resist degradation due to their strong C-F bonds and persist in the environment. Interestingly, the addition of graphene to the Ag nanoparticles greatly enhanced the SERS intensity of the perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) molecules under basic conditions (pH ~9) compared to that of fluorescein and rhodamine. We were able to successfully detect SERS spectra from nano- and pico-molar quantities of PFOA and PFOS respectively, thereby demonstrating the future viability of using our SERS sensors in the environment for ultrasensitive detection of contaminants like PFAS.

(SPEC-12.4) A Non-Lithographic Universal Method to Fabricate Surface Enhanced Raman Scattering Substrates on Different Materials

Ahmed Yousef Fouad Mahmoud¹, Alexandra Teixeira¹, Maria Silva¹, Francisca Guedes¹, Martin Lopez-Garcia¹, Sara Abalde-Cela¹, Lorena Diéguez¹; ¹*The International Iberian Nanotechnology Laboratory (INL)*

Here, we present an inexpensive, non-lithographic, green method to fabricate reproducible and easy-to-use SERS substrates.

Surface-enhanced Raman scattering (SERS) spectroscopy is an ultrasensitive analytical technique that can provide a detection limit down to the single-molecule level and specific vibrational fingerprints. A SERS substrate is a pivotal component in any SERS measurement, where the Raman spectral intensity of the molecule or molecules of interest is enhanced by many orders of magnitude by electromagnetic, chemical, and/or resonance enhancement mechanisms. However, the development of SERS substrates is constantly challenged by their reproducibility, cost, sustainability, and applicability.

Here, we present a green non-lithographic in-situ method to fabricate SERS substrates on different materials. Our approach is based on functionalizing various surfaces using polydopamine film first. Then, the polydopamine-functionalized surfaces are further used as a scaffold on which various plasmonic nanostructures can be deposited and grown using different protocols. We will show how we can systematically tune the plasmonic behavior and optimize the SERS performance of these substrates by varying experimental parameters. Using this method, we were able to in-situ fabricate SERS substrates on paper-like materials, glass, plastics, and in microfluidic devices with basic lab equipment or even without any equipment at all. Some environmental and biomedical applications of these substrates will be presented to show their broad spectrum of applications. We believe that fabricating reproducible, inexpensive, sustainable, and easy-to-fabricate SERS substrates can democratize the applications of SERS in many fields.

(SPEC-12.5) Controlled Citrate Oxidation on Gold Nanoparticle Surfaces for Improved SERS Analysis of Carboxylic and Phenolic Pollutants in Water

Haoran Wei¹, Hanwei Wang¹; ¹*University of Wisconsin-Madison*

Oxidative decomposition of the citrate layer promotes SERS analysis of low-affinity pollutants.

Surface-enhanced Raman spectroscopy (SERS) provides an ultrasensitive, fast, and inexpensive method for organic micropollutant analysis, but its applications are limited by the low affinity of most organic micropollutants towards plasmonic nanoparticle surfaces. Particularly, the citrate layer on gold nanoparticle (AuNP) surfaces exerts strong resistance to ligand exchange and prevents carboxylic and phenolic pollutants from entering SERS “hot spots”. In this study, we aim to extend the application of SERS to the low-affinity carboxylic and phenolic pollutants by oxidative decomposition of citrate layer on AuNP surfaces. The kinetics of citrate oxidation were carefully controlled using sulfate radicals that were slowly released from peroxydisulfate photolysis, which guarantees both the stability of AuNP colloid and generation of a high density of SERS “hot spots” for pollutant analysis. *In situ* Raman spectroscopic monitoring demonstrates that citrate is first oxidized to di- and monocarboxylate acids and subsequently displaced by guest ligands. This oxidation-induced ligand exchange has been applied for SERS analysis of various low-affinity organic micropollutants, including monochloro-substituted carboxylates and phenols, as well as a widely used herbicide – 2,4-dichlorophenoxyacetic acid. This study substantially broadens the library of organic micropollutants for label-free SERS analysis and advances SERS towards a holistic analytical tool for water quality monitoring.

22SPECIAL13: New Platforms and New Applications

(SPEC-13.1) A New Hand-held FT-IR Spectrometer for Field-based Identifications of Vapor Phase Threats

David W. Schiering¹, John Seelenbinder¹, Gregg Ressler¹; ¹*RedWave Technology*

This work describes the first handheld FT-IR spectrometer for in-field identification of vapor phase threats

For responders in the field, toxic vapors represent the most serious threats faced. Several instrumental methods are used to analyze vapors in the field. Many of these technologies suffer from a lack of specificity, limitations on the number of gases that can be detected, high cost, or a lack of portability. A gap exists for a highly portable, cost-effective vapor identifier used for in-field identifications of potential vapor phase threats to public health and safety. The specificity of infrared spectroscopy allows the identification of a large library of vapor phase compounds with high probability of identification. We have developed a new hand-held FT-IR spectrometer designed specifically for use by responders and sleuths, in the field. The spectrometer employs a double pendulum interferometer operating at 4 cm⁻¹ spectral resolution and requires no user alignment. A small pump draws

sample into a 2 meter, low volume gas cell. The instrument weighs 5 lbs and operates on batteries for more than 3 hours. The on board IR spectral library contains 5600 vapor phase entries. The instrument can be operated in a discreet or continuous sampling, identification modes. We will present an overview of the design and technology employed in this new, miniaturized FT-IR spectrometer.

(SPEC-13.2) Waveguide-Enhanced Raman Spectroscopy for Detection of Chemical Vapors

Erik D. Emmons¹, Phillip G. Wilcox¹, Kevin Hung², Erik Roese¹, Ashish Tripathi¹, Jason Guicheteau¹, Ethan Luta³, Benjamin Miller³, Matthew Yates³, Nathan Tyndall⁴, Todd Stievater⁵; ¹*US Army DEVCOM Chemical Biological Center*, ²*Hung Technology Solutions*, ³*URMC*, ⁴*Naval Research Laboratory*, ⁵*Naval Research Laboratory*

Waveguide-enhanced Raman spectroscopy is used to detect hazardous trace chemical vapors.

Detection of threat materials is an important capability for the military and homeland security to protect soldiers and civilians. Waveguide-enhanced Raman spectroscopy (WERS), a photonic integrated circuit sensing methodology, is being developed for field detection of materials related to chemical warfare agents, explosives, and narcotic threats. In WERS, waveguides are used to tightly confine the excitation light over a long path length, leading to large signal levels from molecules present in the evanescent field just above the waveguide. In the present work, low-fluorescence silicon nitride spiral waveguides with path lengths of tens of millimeters are used to obtain high signal levels with near-infrared excitation (785 nm and 1064 nm). Compact single-mode-fiber-coupled spectrometers with high sensitivity are being utilized for detection of the Raman scattered light. Thermoelectrically cooled CCD or InGaAs detectors (-15 °C) provide low noise and high quantum efficiency spectral measurement. Performance comparable to that obtained with large benchtop spectrometers is observed. The spiral waveguides are coated with functionalized polymer sorbents suitable for concentrating relevant classes of vapor materials in the evanescent field of the waveguide. The sorbents are deposited using piezoelectric microdispensers to allow for controlled deposition of thin films without the need for spin-coating. Raman chemical imaging microscopy is used to characterize the uniformity of the sorbent polymers on the waveguides. Library spectral matching can be used in combination with the selectivity of the sorbent materials to provide discrimination of the materials absorbed by the polymer coatings. The ultimate objective is development of a prototype handheld WERS sensor system suitable for defense and security applications in the field. WERS development and spectral measurements will be presented.

(SPEC-13.3) Time-gated Raman spectroscopy for Process Analyses in Downstream Purification Process of Monoclonal Antibody

Amuthachelvi Daniel¹, Mari Tenhunen¹; ¹*Timegate Instruments Ltd*

Time-gated Raman technology aims to fill the void for monitoring the purification process of mAb.

Downstream process, a multi-step process designed for product recovery and purification, is a continuous manufacturing procedure requiring Process Analytical Technology (PAT). In 2004, the US Food & Drug Administration (FDA) issued the Guidance for Industry PAT, titled, 'A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance'. This framework emphasizes the importance of monitoring and thereof controlling the bioprocess to produce high-quality biologics. Spectroscopic methods can be effectively employed to monitor multiple Critical Quality Attributes (CQAs) with little to no sample preparation. Raman spectroscopy is one of the preferred spectroscopic techniques because of its sensitivity and low water signal. Despite these advantages Raman spectroscopy has limited application in downstream process due to the broad fluorescence signal. Time-gated Raman spectroscopy has resolved this impediment by detecting the Raman scattered photons before the emission of fluorescence. At-line time-gated Raman spectroscopy was used in this work for monitoring monoclonal antibody titer and the antibody aggregate amount in samples obtained from different stages of recovery and purification process.

(SPEC-13.4) **Fluorescence-enhanced Photothermal Infrared Spectroscopy**

Craig Prater¹; ¹*Photothermal Spectroscopy Corp*

Sub-micron spatial resolution infrared spectroscopy using fluorescence-detected IR absorption provides enhanced specificity, localization, and sensitivity

We have developed a novel optical microscope-based platform for performing simultaneous and co-located fluorescence microscopy and infrared spectroscopy using Fluorescence-Enhanced Photothermal Infrared (FE-PTIR) spectroscopy. Based on the Optical Photothermal Infrared (O-PTIR) approach, FE-PTIR provides enhanced photothermal sensitivity along with the exquisite selectivity of fluorescence labeling techniques. In the FE-PTIR approach, a fluorescently labeled sample is simultaneously illuminated by pulses of infrared radiation and fluorescent excitation light. When the wavelength of the IR pulses is tuned to a molecular vibration of fluorescently labeled molecules, the absorbed heat causes a modulation in the amount of fluorescent light emitted from the fluorophores. The strong temperature dependent fluorescence emission efficiency of common fluorophores, around of $\sim 1\%/^{\circ}\text{C}$ provides $\sim 100\text{X}$ increase in photothermal sensitivity over the intrinsic photothermal response of most biological materials ($\sim 0.01\%/^{\circ}\text{C}$). Fluorescence localized IR spectra can be created by detecting changes in fluorescence emission as a function of IR wavelength. Simultaneous fluorescence and IR absorption images can be created by mapping the DC fluorescence intensity and the modulation of that fluorescence in response to IR radiation at one or more wavelengths. FE-PTIR thus allows the IR spectroscopic analysis of specifically labeled regions of biological cells and tissue, for example to study conformational stages of a specifically labeled class of target proteins. FE-PTIR can enable correlative fluorescence/vibrational spectroscopy analysis for example associate with protein misfolding associated with neurodegenerative diseases.

FE-PTIR was independently by three groups developed by three groups, including the presenting author(1), and the labs Prof. Ji-Xin Cheng at Boston University (2) and Prof. Garth Simpson at Purdue. (3) This presentation will share details of a newly developed O-PTIR instrument that supports wide-field FE-PTIR and other spectroscopic and imaging capabilities.

1. Prater C. Fluorescence enhanced Photothermal Infrared Spectroscopy and Confocal Fluorescence Imaging. U.S. provisional patent application No. 63/054,167. 2020.
2. Zhang Y et al. Fluorescence-Detected Mid-Infrared Photothermal Microscopy. *Journal of the American Chemical Society*. 2021;143(30):11490-9.
3. Li M et al. Fluorescence-Detected Mid-Infrared Photothermal Microscopy. *Journal of the American Chemical Society*. 2021;143(29):10809-15. doi: 10.1021/jacs.1c03269.

(SPEC-13.5) Spectroelectrochemistry: more than just the sum of its parts
Sergey Shilov¹; ¹*Bruker Optics*

A new accessory for the study of electrochemical reactions

Electrochemistry is a powerful tool for probing and controlling the chemical state of molecules. However, Electrochemistry on its own does not provide information about the chemistry behind the processes under study. The combination of electrochemistry with Fourier Transform infrared spectroscopy bridges this analytical gap. Each molecule has a unique infrared signature providing great specificity in the studies of the molecular change during electrochemical reactions in an addition to the electrochemical response of the studied media.

Spectroelectrochemistry can be applied for investigations of electrolytes or reactions at electrode surfaces. IR reflection-absorbance spectroscopy (IRRAS) set-up is used for the studies of the electrolyte and the electrode surface while ATR configuration is used to analyze the electrode surface without the strong influence of the electrolyte. New accessories for both configurations will be presented. An overview of the experimental electrochemical tools will be presented. In addition, optimization of the spectroelectrochemical setup and details of communication between the potentiostat and the FTIR spectrometer will be discussed. Examples of applications will include electro-oxidation of metal-organic complexes, alcohols, and glycerol.

22SUNKEY01: Keynote Session

(SUN-01.1) The Future of Space Exploration: Earth-based, Deep Space-based, Robotic and Human

Amanda R. Hendrix¹; ¹*Planetary Science Institute*

The role of analytical chemistry and spectroscopy has been crucial to our advances in space exploration over the past decades. In this presentation, I will cover some highlights of relevant solar system studies and discoveries, and look ahead to the future.

Studies using Earth-based spectroscopic datasets (e.g., ground-based telescopes and the Hubble Space Telescope, HST) have been significant for systematic surveys and also initial discoveries that have driven later deep-space missions. For instance, the discovery of possible cryovolcanic plumes at Europa using HST data was critical in guiding requirements for the upcoming Europa Clipper mission.

Sample return missions - e.g., Apollo at the Moon, the Stardust mission, Hayabusa and OSIRIS-REx missions to near-Earth asteroids - are fundamental for our understanding of the history of the solar system and planetary processes, and allow us to utilize the wealth of sophisticated Earth lab-based analytical chemical techniques. Currently the Perseverance mission is collecting samples from Mars that will be groundbreaking for our understanding of the history and habitability of that planet.

The chemical analyses using data from the Cassini mission at the Saturn system have revealed the ocean worlds of Enceladus and Titan. Exciting discoveries include ethane- and methane-rich lakes on the surface of Titan and a plume of water and organics emanating from Enceladus' sub-surface ocean. We now know that Enceladus' ocean is a habitable environment. Is it in fact inhabited? The organic-rich atmosphere and surface of Titan will be further studied by the Dragonfly mission. Could Titan be a self-sustainable destination for future humans?

Looking ahead to upcoming missions such as JWST, Clipper at Jupiter's moon Europa and Dragonfly at Titan, and to future missions at Uranus and Enceladus, we look forward to an increased understanding of ocean worlds and to the critical use of analytical chemistry to unlock the exciting secrets of the final frontier.

Monday, October 3, 2022

Oral Presentations

22ART01: Student Research in Archaeological Chemistry

Chair: John Murray

(ART01.1) Vessels and their Residues: Exploring Nuances in the Diverse Scapes of South-Asia

Ahana Ghosh¹; ¹*Indian Institute of Technology*

This research explores the diverse scapes of South Asia and delves into the use - ware analogy of the Subcontinent, especially looking into the residues inside the vessels. The climatic and topographical diversity of South Asia actuates distinct traces of altered uses of vessels. However, the culinary landscapes of South Asia encourage varied foot trails throughout the Subcontinent. Food archaeology is still in an embryonic stage in South Asia, as the previous studies primarily focused on nuances in an individualistic way, especially

leaning on the aspects like methodological pursuits and often peeking into the ethnographic abstractions. Residue analysis is currently developing as a significant tool in South Asian archaeology concerning paleo-dietary conjectures, use-ware analysis, and multiple ecological assertions. Due to the diverse climatic pattern of South Asia, lipid preservation in archeological ceramics varies regionally, and finally, mingling with possible contaminants leaves an adverse effect on the obtained analytical results. The foodscapes of the regions are characterized by resilient dietary patterns strongly influenced by humid and dry climatic conditions. This research significantly bifurcates from the previous researches and wields food archaeology and food ethnography to peek into the culinary traditions throughout the diverse regions. Further, this research examines the biomolecular components lying in the organic residues from the Harappan settlements of Gujarat and Chalcolithic settlements of coastal-Eastern India within ceramics used by the inhabitants of these sites. This will aid in further understanding of their obscure economic and subsistence practices associated with the more extensive proto-historic cultural and technological traditions of the settlements. In the above research, stress has been given to performing organic residue analysis on several ceramic samples from the burial and habitational Harappan settlements of Gujarat like Dholavira, Shikarpur, and Eastern-Indian coastal settlements like Erenda for understanding the relationship between the Harappan ceramic typology and vessel use patterns from Early to Mature Harappan period in this settlements. Finally, it also proposes a few distinct sampling methods for residue analysis recently developed for the diverse South Asian landscapes.

(ART01.2) Geochemical Data and Geospatial Methods: Characterizing Obsidian Use and Movement in Late Pleistocene Eastern Africa

Sydney E. James¹; ¹*Arizona State University*

The semi-recent introduction of technologies such as X-Ray Fluorescence spectrometry to archaeology has been largely beneficial for the sourcing of materials via geochemical characterization. As a result, studies of obsidian transport during the Late Pleistocene of eastern Africa have been largely productive for reconstructing patterns of raw material procurement and movement and may offer valuable insight into the development of social networks. Due to a limited sample, however, these studies are often descriptive of particular sites and related explicitly to material provenance and transport distance, and little is known about the factors influencing procurement. Archaeological and experimental studies from other regions and time periods indicate that obsidians may have been preferentially selected for quality and/or symbolic properties. A bottom-up understanding of the nature of obsidian movement across the dynamic volcanic landscapes of the Rift Valley is therefore needed before inferences about their relation to social networks can be made. Using quantitative geospatial methods in conjunction with available lithic provenance data in obsidian-bearing later Middle and Late Pleistocene archaeological sites, this research seeks to answer two questions: 1) What factors mediated obsidian use during Marine Isotope Stages 5-4 (130 – 58ka) in eastern Africa? And 2) At what point is the change in obsidian use - from strictly utilitarian to culturally significant - visible in the archaeological record? Results suggest some archaeological occurrences of obsidian movement over 50 km cannot be explained by proximity alone. Because multiple sources are often utilized within a single site, the

hypothesis that humans procured obsidian through multiple different pathways remains to be tested. The methods and results presented here are needed to build testable models to study landscape use and social connectedness in early Late Pleistocene *Homo sapiens* populations.

(ART01.3) **pXRF as a Method to Identify Ochre Residues on Archaeological Ostrich Eggshell Fragments**

Hannah M. Keller¹, Ellery Frahm¹, Jessica C. Thompson¹; ¹*Yale University*

Ostrich eggshell (OES) (*struthio* spp.) is a frequently reported find at many sites in the African and Asian archaeological record. Ostrich eggs are an important nutritional, technological, and social resource, as the eggshell can be fashioned into flasks and beads. Thus, OES fragments recovered from the site may represent either flasks or the remnants of cooked eggs. Moreover, although there is ethnographic evidence for use of pigments either to decorate the cuticle surface or transported within the flasks, no one has investigated unmodified fragments for pigments. This has the potential to inform if the egg was carried in a bag or by hand and acquired pigments incidentally (color predominantly on the outside), if it was used as a container for pigments (color predominately on the inside), or if pigment traces were acquired post-depositionally from the sediment (color on both). To test this hypothesis, our study has developed protocols for identifying elemental signatures of ochre applied to OES surfaces through portable X-Ray Florescence (pXRF) and compared these signatures to pigments identified by a Scanning Electron Microscope (SEM). While the SEM is widely used in archaeological studies, the pXRF is cheaper and more portable option, making it more accessible to researchers. We prepared modern OES fragments by coating the surfaces in a mixture of ochre pigments and lard and tested the Fe content after subjecting them to washing, burning, and burial for six months. Our experimental study demonstrates that residues persist after these taphonomic processes, as does an increased Fe signature in the SEM and pXRF. We applied this methodology to two archaeological assemblages in eastern Africa, to test hypotheses of flask manufacture and use among foragers of the late Pleistocene (~129,000-11,000).

(ART01.4) **Developing an Empirical Calibration for Elemental Characterization and Sourcing of South African Silcrete with pXRF**

John K. Murray¹, Jayde N. Hirniak¹, Andrew M. Zipkin²; ¹*Arizona State University*,
²*Eurofins EAG Laboratories*

Geochemical provenience studies of toolstone provide archaeologists with a vehicle for reconstruction of the social networks, mobility patterns, and possibly exchange networks of ancient human populations. However, sourcing stone artifacts can be problematic because methods are often destructive and can damage artifacts. Additionally, many instruments are not readily available in the countries where archaeologists work, necessitating the export of artifacts for analysis. Therefore, this research project aims to address these issues for silcrete, a significant class of toolstone in multiple regions, by developing an empirical calibration for portable X-ray Fluorescence (pXRF) analysis. pXRF is a non-destructive and field-portable technique for measuring the elemental composition of a material. To

effectively use pXRF to source raw materials, the calibration settings must be optimized for the target material. Currently, there is no empirical calibration for silcrete rock from South Africa. To create this calibration, an experimental reference collection of geochemical compositions will be developed from three sources of silcrete in South Africa that have been analyzed with solution inductively coupled plasma-mass spectrometry. The results of this project will provide archaeologists with an empirical silcrete calibration for accurately characterizing and potentially sourcing silcrete artifacts using an approach that is non-destructive, portable, and relatively inexpensive.

(ART01.5) “It Starts Down Below”: A Preliminary Study of Pollution Levels in Animals from the Southern Carpathian Bronze Age with ICP-MS

Iride Tomazic¹, Amy Nicodemus², John O’Shea¹; ¹*University of Michigan*, ²*University of Wisconsin La Crosse*

Anthropogenic pollution culminated in the 20th and 21st centuries. As a result, a more significant scientific focus has been devoted to understanding pollution's levels, effects, and long-term consequences on the environment, people, and animals in ecological, medical, and public health studies. However, while current pollution levels are alarming, they result from continuous environmental neglect. Furthermore, research from the Greenland ice cores and local lakes and bogs shows pollution's deep history. In Europe, pollution spikes have been recorded dating to the Bronze Age, the Roman period, and the Industrial Revolution, thus putting archaeology in a unique position to investigate its long-term consequences. However, little attention to pollution has been dedicated in archaeology. This lack of study is mainly due to the skepticism around the accurate representation of heavy metals in archaeological material as a result of diagenetic processes. However, this doubt might be resolved with systematic sampling and methods. In this study, we present preliminary results of ICP-MS and LA-ICP MS on animal second and third molar mandibular enamel from three Bronze Age sites (2400 - 1500 BC) in the Southern Carpathian basin. The analysis results showed that levels of heavy metals vary by animal species and habitat, thus offering an opportunity to investigate questions of pollution in buried archaeological material.

22ATOM01: LA-ICP-MS

Chair: C. Derrick Quarles Jr.

(ATOM-01.1) Dual fs-LIBS & fs-LA-ICPTOFMS System (Not Simultaneous) for Fast and High Dynamic Range Micro-Analysis: Pros and Cons.

Jorge Pisonero¹, Cristina Méndez-López¹, Cristian Soto, Jaime Orejas, Ana Méndez, Antonia Cepedal, Nerea Bordel¹, Lukas Schlatt², Phil Shaw²; ¹*University of Oviedo*, ²*Nu Instruments*

Multi-elemental analysis of solid samples using rapid and direct methods is a key point in the development of analytical sciences. Over the years, techniques based on Laser Ablation such as Laser Ablation Inductive Coupled Plasma Mass Spectrometry (LA-ICP-MS) and Laser Induced Breakdown Spectroscopy (LIBS) have been widely used, rapidly evolving into well-established, mature powerful tools for direct, highly sensitive and high lateral

resolution analysis in numerous fields such as geology, biology, metallurgy, or environmental sciences [1]. Significant research and advances continue to thrive for achieving the fastest, most accurate and efficient analysis, such as those focused on improvements such as low dispersion setups and cell geometries for the finest control of aerosol trajectories [2].

Femtosecond laser ablation reduces melting effects around the ablated area as well as fractionation effects. The combination of a fast-response femtosecond laser ablation unit and novel ICP-TOFMS technology provides one of the top-most interesting analytical methods for high spatial resolution determination -imaging- in samples of different matrices and nature. Moreover, the possibility of extracting the fs-laser beam out of the ablation unit, for LIBS analysis, provides a complimentary tool for the determination of major elements or those non-accessible to ICP-TOFMS..

In the present work, the analytical capabilities of a NWRfemto laser both for LIBS studies and coupled to a Nu Vitesse ICP-TOFMS, are evaluated. In particular, the potential of this novel ICP-TOFMS is investigated for fast elemental imaging applications and for single shot multielemental analysis. Additionally, the option of configuring the attenuation of the ion signals at different levels in multi-segmented methods allows for the analysis of highly abundant isotopes next to other low abundant/trace ones, hence boosting the dynamic range of LA-ICP-MS at the low micrometric scale.

[1] J. Pisonero, D. Bouzas-Ramos, H. Traub, B. Cappella, C. Alvarez-Llamas, S. Richter, J. C. Mayo, J. M. Costa-Fernandez, N. Bordel, N. Jakubowski, *J. Anal. At. Spectrom.*, 34, 655-663 (2019).

[2] C. Neff, P. Becker, D. Günther, *J. Anal. At. Spectrom.*, 37, 3, 677 – 683 (2022).

(ATOM-01.2) **Recent Developments for In Situ Sr Isotope Ratios and Rb/Sr Geochronology by LA-ICP-MS/MS**

Alicia Cruz-Uribe¹, Cemil Arkula; ¹*University of Maine*

Recent advances in tandem mass spectrometry (ICP-MS/MS) have facilitated the inline separation of isobaric interferences using reaction cell chemistry. This enables the measurement of radiogenic isotopes in systems in which the parent and daughter products are isobaric (i.e., ⁸⁷Rb decays to ⁸⁷Sr, ¹⁷⁶Lu to ¹⁷⁶Hf), thereby drastically reducing sample preparation and analysis time. Here we separate ⁸⁷Rb from ⁸⁷Sr using SF₆ as a reaction cell gas in the octopole reaction cell of an Agilent 8900. We measure ⁸⁵Rb on-mass and calculate the corresponding ⁸⁷Rb, and measure ⁸⁶Sr and ⁸⁷Sr mass-shifted as SrF (at masses 105 and 106, respectively) to determine the ⁸⁷Sr/⁸⁶Sr and ⁸⁷Rb/⁸⁶Sr ratios. This enables the determination of *in situ* isochron dates using the Rb/Sr system in geologic materials by laser ablation (LA) ICP-MS/MS. Use of the reaction cell also has the benefit of eliminating isobaric interferences on ⁸⁷Sr from doubly charged rare earth elements (¹⁶⁴Yb⁺⁺, ¹⁶⁴Er⁺⁺), which is important for analysis of Sr isotope ratios in minerals that incorporate significant quantities of rare earth elements (i.e., apatite, feldspars).

High-Rb minerals such as micas and feldspars are some of the most common minerals in the Earth's crust, and have traditionally been dated using Rb/Sr by conventional bulk analysis techniques. *In situ* measurement of these phases via laser ablation allows the textural context to be preserved during analysis, opening up a wealth of possibilities to examine the geochronologic history of a wider range of rock types and minerals than current accessible techniques (i.e., U/Pb in zircon and other phases). The wide spread in Rb/Sr ratios in natural mica samples gives exceptional leverage on an isochron diagram, resulting in date determinations with uncertainties of a few percent. Seventeen spot analyses of biotite from the LaPosta Pluton give a Rb/Sr isochron age of 92.7 ± 3.1 (2s; MSWD=0.84) Ma and an initial $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.7049 ± 0.0031 , which is consistent with the traditional bulk Rb/Sr age of 93.8 ± 2.5 Ma (Walawender et al. 1990).

(ATOM-01.3) Elemental Histology: New Frontiers in LA-ICP-TOF-MS

Keith MacRenaris¹, Andrew Crawford, David Zee, Qiaoling Jin, Thomas O'Halloran;

¹*Michigan State University*

Although laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been used since the 1990s for bioimaging applications, recent technological advances have allowed for more high-throughput elemental mapping representing a watershed moment for inorganic physiology and opening the doors for new discoveries in biology and medicine. This is attributable to faster laser repetition rates, improved washout times via new ablation cell designs, and nearly simultaneous full mass spectrum coverage via time-of-flight mass spectrometers (TOF-MS). In this talk, I will discuss our current projects focusing on the determination of elemental content and localization at the subcellular, cellular and organismal level using our state-of-the-art ESL Bioimage 266 laser ablation system coupled to the Tofwerk S2 ICP-TOF-MS. Through these analyses we are discovering the intricate interplay in inorganic regulatory networks from such diverse areas as developmental biology, microbial metal homeostasis, metal dysregulation in host-pathogen interactions as well as monogenic diseases such as Wilson's disease and hemochromatosis. In addition, we will discuss current challenges in sample preparation, calibration, and data analysis with a focus on multi-modal elemental imaging combining synchrotron-based X-ray fluorescence microscopy (XFM), LA-ICP-TOF-MS, and photoacoustic microscopy (PAM) to show how these tools can lead to a broader understanding of metals role in systems biology and human pathology.

(ATOM-01.4) Determination of Neurodegeneration-related Cytosolic Proteins in Individual Human Epithelial Cells by LA-ICP-MS Using Novel Matrix-Matched Standards and Metal Nanoclusters as Immunoprobosc-Labels

Ana Lores Padin¹, Beatriz Fernandez¹, Montserrat García², Héctor Ganzález Iglesias³, Rosario Pereiro¹; ¹*University of Oviedo*, ²*Instituto Oftalmológico Fernández-Vega*, ³*IPLA-CSIC*

Human metallothionein 2 (MT2A) and apolipoprotein E (APOE) are two prominent proteins involved in protecting the retinal pigment epithelium (RPE) from oxidative stress and subsequent inflammation. Both conditions could lead to the development of age-related

macular degeneration (AMD), the leading cause of blindness in the ageing population [i]. Thus, understanding the effects of pro-inflammatory cytokines on both proteins expression in RPE cells may provide meaningful insight into AMD progression. However, it is well-known that the study of biological processes in cells is not straightforward due to cellular heterogeneity and the fact that they can respond differently to external stimuli [ii]. Thus, there is a need to develop new analytical methodologies that provide information at the cellular level.

In this regard, laser ablation (LA) coupled to ICP-MS is a promising complementary alternative to liquid nebulization *single cell*-ICP-MS for the analysis of individual cells. Furthermore, not only the analysis of elements naturally present in the cells can be tackled but also specific biomolecules can be detected through the combination of LA-ICP-MS with adequate metal-labelling strategies. LA-ICP-MS allows the subcellular resolution mapping of target biomolecules as well as a non-size-bias cell introduction into the ICP.

In our research, the sequential quantification MT2A and APOE in individual human retinal pigment epithelial (HRPEsv) cells subjected to inflammation with cytokine Interleukin-1 α (IL-1 α) by LA-ICP-MS is carried out. A single biomarker strategy using well-characterized Au nanoclusters (AuNCs) as specific antibody labels is performed. Additionally, considering the lack of suitable and commercially available reference materials for LA-ICP-MS quantification in biological samples, in this work we introduce a novel matrix-matched calibration strategy which fully mimics the sample matrix of the cells. Thus, HRPEsv cells (the same cell line as the samples) supplemented with suspensions containing nude AuNCs were prepared to generate single-cell laboratory standards (HRPEsv@AuNCs cells).

[i] M. Fleckenstein, T.D.L. Keenan, R.H. Guymer, U. Chakravarthy, S. Schmitz-Valckenberg, C.C. Klaver, W.T. Wong, E.Y. Chew, Age-related macular degeneration, *Nat. Rev. Dis. Primers* 7 (2021) 32. <https://doi.org/10.1038/s41572-021-00272-3>

[ii] P.E. Oomen, M.A. Aref, I. Kaya, N.T.N. Phan, A.G. Ewing, Chemical analysis of single cells, *Anal. Chem.* 91 (2019), 588-621 <https://doi.org/10.1021/acs.analchem.8b04732>

(ATOM-01.5) **Elemental Distribution in Shark Teeth Using High-Speed LA-ICP-MS Imaging**

C. Derrick Quarles Jr.¹, Benjamin T. Manard², Christopher Hintz³, Alicia Cruz-Uribe⁴, Joseph Petrus⁵, Cole R. Hexel²; ¹*Elemental Scientific, Inc.*, ²*Oak Ridge National Laboratory*, ³*Savannah St. University*, ⁴*University of Maine*, ⁵*Elemental Scientific Lasers*

Quantifying the chemical composition of fast-growing hard tissues in the environment can shed valuable information on ecosystem evolution both current and prehistoric. Changes in chemical composition can be correlated with environmental conditions and can provide information about the life history of organisms. Sharks can lose 0.1 to 1.1 teeth per day,

depending on species, which offers a unique opportunity to record environmental changes over a short period of time.

In this work, high-speed imaging was performed using laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) utilizing a two-volume laser ablation chamber (TwoVol3). Three different shark species (tiger, sand tiger, and hammerhead) were investigated for elemental distribution changes across the dentine, enamel, and root regions of the teeth. Of particular interest is the distribution of Mg, Mn, Zn, and Sr, which helps researchers understand more about the environmental conditions endured by sharks. In the past these types of measurements have been performed by bulk acid digestion ICPMS, x-ray spectroscopy, or electron microprobe, which suffer from lack of spatial information or long analysis times. Utilizing the high-speed imaging capabilities of the imageGEO193 allows for high-resolution elemental mapping in a timely manner, making it possible to look at larger data sets.

22IR08: Advances in Vibrational Spectroscopy for PAT and Process Chemistry

Chair: John Wasylyk

Co-Chair: Mike George

(IR-08.1) In Situ Monitoring of Amorphous Solid Dispersions Using Low Frequency (THz) Raman Spectroscopy

Alison Nordon¹, Pattavet Vivattanaseth¹, Magdalene Chong¹, Elke Prasad¹, Gavin W. Halbert¹, John Robertson¹, Catriona McFarlan¹; ¹*University of Strathclyde*

THz Raman spectroscopy and multivariate curve resolution can characterise solid forms in hot melt extrusion

Off-line analysis techniques can be used to characterise solidified extrudates from hot-melt extrusion (HME) processes. However, by the time a sample is presented for off-line analysis, the sample may not be representative of the melt mixture as it may have changed upon cooling. Low frequency (THz) Raman spectroscopy can be used to characterise the form of solid dispersions in the melt mixture *in situ*. Calibration-free methods such as multivariate curve resolution (MCR) can be used to resolve mixture spectra into pure component contributions using an iterative optimisation within a set of constraints. The outputs are the pure spectra and concentration profiles for each component. This study investigates the solubility limit and changes in the molecular arrangements of mefenamic acid (MFA) in a matrix of sorbitol and Soluplus with THz Raman spectroscopy and determines the concentration ratio of amorphous and crystalline states.

Mixtures of 10 – 50% (w/w) MFA, sorbitol and Soluplus were extruded at different temperatures and *in situ* THz Raman spectra were acquired of the melts. The melts containing amorphous and crystalline forms of MFA were readily distinguishable, with characteristic peaks at 29 and 44 cm⁻¹ for the crystalline form of MFA. MCR was applied to the matrix of spectra acquired; the two pure component spectra obtained were comparable to the *in situ* spectra acquired from the extrusion of 10% MFA at 140 °C and 50% MFA at 130 °C, and hence were assigned as amorphous and crystalline components, respectively.

The relative concentration profiles of the two molecular arrangements were also obtained. The concentration profiles of the crystalline content, normalised to that of 50% MFA extruded at 130 °C, were then plotted as a function of MFA content and extrusion temperature. The most crystalline samples were obtained at high MFA concentrations and low extrusion temperatures.

This study has demonstrated that a combination of THz Raman spectroscopy and MCR can be used to characterise a ternary melt mixture. Two forms of MFA and their relative concentrations in a mixture could be identified using the method. This information can be used in the development and monitoring of extrusion processes.

(IR-08.2) Driving Sustainable Research by Maximizing Spectroscopy and Spectrometry Tools

Robert Wethman¹, John M. Wasylyk¹, Ming Huang¹, David Fenton²; ¹*Bristol Myers Squibb*, ²*Rowan University*

Multiple analytical tools are used for sustainable analyses

Over the last several decades, tremendous advances have been made in sustainable chemistry, with a heavy focus on the synthetic arena. Complementary, are the more recent advances in enhancing green approaches in analytical chemistry for supporting synthesis at all levels from discovery to scale-up to manufacturing. The recent analytical focus has been on the prevention of waste generation; safer solvents and auxiliaries; design for energy efficiency; all aided by the application of traditional and cutting-edge development of spectroscopy-based analytical methods. The application of vibrational-spectroscopy-based techniques, coupled with multi-component analysis greatly enhances process knowledge and control without adding additional non-sustainable assays. This includes the careful selection of the most appropriate techniques based on experience, pre-screening, and greenness. The limitations of vibrational-spectroscopy can be address in certain cases by in-line mass spectrometry without the need for any pre-treatment including gas column separation. Sampling by mass spectrometry can occur through the headspace of reactors and be used to ensure scrubber efficiency as well as for drying end-point determinations. We will show examples of how we select the appropriate analytical technique and simplify sampling, in order to generate data that provides enhanced process knowledge, as well as drive the sustainable analytical approach into the manufacturing environment.

(IR-08.3) Use of Vibrational Spectroscopy in Cosmetic Science and Claims Substantiation

Samuel Gourion-Arsiquad;

(IR-08.4) Self-Optimising Flow Reactors for Multi-objective and Multistep Process Development

Richard A. Bourne¹, Adam Clayton, John Blacker, Tom Chamberlain, Richard A. Bourne¹, Nik Kapur; ¹*University of Leeds*

This talk will focus on the development of automated continuous flow systems. In particular, recent research on self-optimising systems where the reactor and its process control instrumentation become an autonomous unit into which the reactants are pumped, and from which products emerge with optimized. This presentation will outline the recent grant ‘Cognitive Chemical Manufacturing’ and the new approaches to synthesis of fine chemicals and pharmaceutical compounds. These automated systems work without human intervention and are capable of very robust experimentation and rapid optimisation of challenging processes. This talk will focus on optimisation of multiple unit operations including optimisation of telescoped reactions and reaction followed by continuous work-up. It will also explore the use of algorithms capable of optimising the trade-off between conflicting objectives such as yield and reactor productivity.

(IR-08.5) **Self-Optimisation of Flow Processes using A-TEEM Spectroscopy**

Ashley Love;

A-TEEM spectroscopy is a powerful optical spectroscopic technique which simultaneously collects the Absorbance, Transmittance and fluorescence Excitation Emission Matrix (A-TEEM) of analytes. The excitation emission matrix acts a “molecular fingerprint” allowing for species which exhibit normally indistinguishable UV/vis absorption and emission characteristics in traditional spectroscopies, to be easily identified. This all can be achieved rapidly, with sensitivity to chemical species down to the parts per billion. The vast amount of chemical information collected with each measurement becomes all the more powerful when combined with chemometrics and multivariate analysis. These approaches allow for not only the deconvolution of spectra aiding analysis, but also the classification and prediction of their chemical makeup. These advantages make A-TEEMs the ideal technique to bring about improvements in the field of reaction self-optimisation, where techniques such as Mass Spectrometry, NMR, GC and HPLC do not provide the ease of use, sensitivity, time resolution and rich chemical information combined, which is available through A-TEEMS. This work utilises A-TEEMS to demonstrate that it can both monitor and optimise chemical reactions in a rapid and precise manner, making it an indispensable tool for reaction self-optimisation. We then deploy this approach to self-optimise photochemical transformations in flow.

22LIBS01: Fundamentals

Chair: Alessandro De Giacomo

Co-Chair: Jonathan Merten

(LIBS-01.1) **Probing LIP-Atmosphere Interaction with Atomic Absorption Spectroscopy**

Jonathan A. Merten¹, Hannah Bariola¹, Shealyn Chestnut¹, Erin Nicholas¹, Shawnda Ethridge¹, Mary Foster¹; ¹*Arkansas State University*

The laser-induced plasma (LIP) is typically studied with atomic emission spectroscopy. Though substantially more complicated, atomic absorption measurements are

also possible and allow absolute measurement of the extent of a given atomic state in the plasma. Since most absorption measurements in the plasma use (semi)coherent probes, their well-defined line of sight allows spatially resolved measurements. Our lab has developed a laser-ablation absorption technique that we call pseudocontinuum source atomic absorption spectroscopy (psCS-AAS). We have used spatially-resolved psCS-AAS measurements to study evolution of the extent, thermodynamics and distribution of material in the LIP as a function of cover gas. Data are presented both at times relevant to the usual thermal emission measurements and at delays greater than 10 microseconds, where thermal emission is negligible. These measurements allow insight into the empirically well-known differences in signal observed when LIP thermal emission measurements are made under different cover gases.

(LIBS-01.2) Comprehensive Diagnostics of LIBS Plumes by Combining Emission and Absorption Spectroscopy

Sivanandan Harilal¹, Elizabeth J. Kautz¹, Mark C. Phillips²; ¹*Pacific Northwest National Laboratory*, ²*University of Arizona*

LIBS – a combination of laser-produced plasma (LPP) and optical emission spectroscopy (OES) - is a well-known analytical tool that uses self-emission from the laser-produced plasma for analyte identification and concentration measurement. OES is also the most used diagnostic tool for various plasmas sources, including LIBS, to measure its physical properties due to its experimental simplicity as a non-intrusive technique. However, there are certain limitations for using OES for LPP characterization, most prominent is that the LPP emits line radiation through electronic excitation only within a certain temporal window during its evolution. For example, at later times of LPP evolution, the plasma temperature drops significantly so that the electronic excitation process becomes weak or nonexistent. The thermal excitation predominates closer to the target in an LPP, while cooler conditions exist at farther distances from the target. Active sensing methods such as laser absorption spectroscopy (LAS) which utilizes the ground or lower level population, are useful for measuring the properties of an LPP at later times of its evolution where the electronic excitation is not favored. Although absorption spectroscopy is a well-established technique for gas sensing, its use for LPP characterization is limited, partly due to its active nature that necessitates the use of a light source such as a laser for probing the absorption by the LPP species. In this work, we used a combination of OES and LAS for the comprehensive characterization of a LIBS plume. Since emission and absorption methods are complementary, by combining these two techniques, fundamental properties of the plasma can be measured at early and late times of LPP evolution as well as from various spatial positions.

(LIBS-01.3) Fundamental Approaches to Broaden the Applications of Commercial Handheld LIBS

Matthieu Baudalet¹, Kristen Livingston¹, Magdalena E. Jackson²; ¹*University of Central Florida*, ²*Rensselaer Polytechnic Institute*

The calibration-free method has mostly been successfully applied to LIBS (CF-LIBS) in laboratory conditions. However, field analysis is increasingly required for the analysis of

several samples where no standards are available (such as anthropology and archaeology). As a result, it is more practical to use a portable, handheld LIBS (hhLIBS) instrument to analyze these materials. This study establishes whether the calibration-free method can be applied to hhLIBS data to successfully measure elemental concentration using CF-LIBS. Demonstrating the ability of calibration-free hhLIBS to serve as an accurate quantitative technique would decrease the need for matrix-matched standards and will broaden the range of analytical applications in fieldwork. Results and also discussion about the fundamental differences between traditional and handheld LIBS plasmas will be the focus on this talk, in order to determine how much broader we can bring quantitative portable LIBS into areas where calibration standards are not available yet.

(LIBS-01.4) Femtosecond and Nanosecond Laser-assisted Surface Processing of Crystalline Silicon

Reji Philip¹, Nancy Verma¹, Nithin Joy¹, Kiliyanamkandi Anoop²; ¹*Raman Research Institute*, ²*Cochin University of Science and Technology*

Femtosecond laser-induced periodic surface structuring is used to create black silicon having variable structural periodicity.

Femtosecond laser-induced periodic surface structuring (fs-LIPSS) is a reliable and unique method for micro-nanoscale texturing and surface functionalization of solid materials. In this work, we investigate the roles of laser pulse duration, wavelength, and background pressure in the fabrication of surface structures on crystalline silicon through a single-step, chemical-free process. 100 fs (800 nm, 400 nm) and 7 ns (532 nm) laser pulses, obtained from Ti:Sapphire and Nd:YAG lasers respectively, are employed for the process. The laser energies are adjusted to be close to the ablation threshold of the target surface. Pulses of fs duration lead to the formation of nanoscale periodic and quasi-periodic patterns on the silicon surface, the periodicity of which is dependent on the laser wavelength. Clean structures free from nanoparticles are obtained when the silicon wafer is irradiated in high vacuum (5×10^{-5} Torr). Irradiation using ns laser pulses produces irregular microscale structures in comparison. A substantial reduction in surface reflectivity, due to surface blackening, is seen in both cases, which is more pronounced with fs texturing. Measured Raman spectra reveal that the crystallinity of the silicon lattice is not affected by the texturing process. Raman spectra further show that texturing using fs laser pulses results in a lower scattering efficiency compared to that using ns laser pulses. Silicon surfaces blackened by fs-LIPSS can have a variety of applications in photovoltaics, silicon photonics, electro-optic devices and sensors.

(LIBS-01.5) Vapor-phase Chemical Speciation and Condensation of Cerium Oxide Nanoparticles

Kate Rodriguez¹, Batikan Koroglu², Joshua Hammons¹, Zurong Dai¹, Kim Knight¹;
¹*Lawrence Livermore National Lab*, ²*LLNL*

Novel flow reactor setup used to track vapor-phase formation and evolution of cerium oxide nanoparticles

Local conditions, such as temperature and oxygen availability, have been shown to have a pronounced effect on the formation and evolution of fallout following a nuclear explosion.

While the behavior of nuclear-relevant materials such as uranium have begun to be explored under a wider range of environments, little is known about the behavior of plutonium. Using cerium as a surrogate, we track the vapor-phase synthesis of cerium oxide nanoparticles created in a plasma flow reactor under conditions of controlled temperature and oxygen fugacity. *In-situ* optical emission spectroscopy is used to detect the presence of atomic, ionic, and molecular species and measure their spectral intensity variations with temperature and oxygen content. We find that the relative rate of gas-phase oxidation of cerium is highly dependent on both the temperature and local redox conditions within the flow reactor, to the extent that doubling the oxygen availability effectively doubles the amount of vapor-phase cerium monoxide at high temperature (>2000 K). Condensed cerium oxide nanoparticles are also collected and analyzed *ex-situ* via transmission electron microscopy, grazing incidence small angle X-ray scattering, and dynamic light scatter techniques to determine their elemental composition, crystal structure, and size distribution. The size and morphology of the condensed nanoparticles is independent of local redox conditions, forming the same crystal type with the same size distribution regardless of oxygen availability. Particle condensation is, however, found to be predominantly driven by temperature effects, with the average particle size increasing as particles are allowed to cool and subsequently aggregate together. Results show that the processes that underpin the vapor-phase chemical speciation of cerium oxide are highly dependent on the local environment (temperature, oxygen fugacity), while the final condensation products are not, and lay the groundwork for extensions into the behavior of plutonium.

22MASS01: Mass Spectrometry and Space

Chair: Theresa Evans-Nguyen

Co-Chair: Jacob Shelley

(MASS-01.1) Universal Liquid Sampling Ionization Mass Spectrometry

Theresa Evans-Nguyen¹, Ashton Taylor¹, Cheyenne Sircher¹; ¹*University of South Florida*

To expand the utility of mass spectrometry in planetary applications, targeting larger molecular weight analytes requires coupling to liquid phase separations such as capillary electrophoresis. Electrospray (ESI) is the default ionization method of choice in laboratory applications but is not amenable to analytes such as nonpolar polyaromatic hydrocarbons which lack a readily ionizable moiety. To access a broad spectrum of analytes, we have developed a universal liquid sampling ionization source comprised of acoustic desorption and atmospheric pressure chemical ionization (APCI). We previously demonstrated signal intensities comparable to ESI for both polar and non-polar species. Literature suggests that the ionization mechanism may be more robust to matrix effects and possibly electrolytic solutions such as those used in CE. In the presented work we will demonstrate our alternative sampling method with prototypical CE solutions and representative biochemical targets relevant to astrobiological investigations.

(MASS-01.2) SILICA (Surface Investigation via Lunar Imaging and Compositional Analysis): A Versatile Lunar Mission Concept

Ricardo Arevalo¹, Ann Parsons, Soumya Ray, Ben Farcy, Mauricio Ayllon-Unzueta, Bret Bronner, Ryan Danell², Adrian Southard¹, Andrej Grubisic¹, Jacob Graham, Cynthia

Gundersen, Julie Llano, Christelle Briois, Laurent Thirkell, Fabrice Colin, Alexander Makarov; ¹University of MD, ²Danell Consulting

The SILICA investigation will characterize the chemistry of lunar volcanics (and their respective source regions), provide insights into the composition of the bulk silicate Moon, and define the organic inventory and resource potential of lunar surface materials. These objectives address a multitude of high-priority science questions captured in the Artemis III SDT Report, the Planetary Science Decadal Survey (2023-2032), and a host of other documents released by the lunar science community.

The payload for this mission concept centers on two cutting-edge technologies: 1) CRATER (Characterization of Regolith And Trace Economic Resources), a laser desorption mass spectrometry (LDMS) instrument leveraging an ultrahigh resolution Orbitrap™ mass analyzer ruggedized for spaceflight; and, 2) BECA (Bulk Elemental Composition Analyzer), a gamma ray spectrometer (GRS) and pulsed neutron generator derived from the design baselined on the Dragonfly mission.

Onboard a commercial lunar lander, CRATER will collect 2D chemical images (500 micron field of view) and 3D depth profiles (sub-micron resolution) of regolith collected near the landing site. A vertical profile will be facilitated by a sample handling system that will provide access to samples collected at the surface, as well as at depths down to 10 cm, enabling investigations into space weathering. By leveraging the mobility of a small lunar rover, BECA will measure the abundances of major and minor rock-forming elements, as well as H, rare earth elements, and heat-producing K, Th, and U across a km-scale transect extending away from the landing site towards an adjacent lithological unit. With the ability to measure subsurface bulk composition non-destructively down to 20 cm depth, BECA will provide a comparative analysis of the landing site and extend the investigation objectives along the rover traverse.

SILICA can be deployed at a variety of landing sites and provide a comprehensive perspective on the composition of the lunar surface across a continuum of spatial scales (from sub-micron to km). Here, we present a flexible concept of operations that highlights the versatility of the investigation, a demonstration of the analytical power and scientific reach of the CRATER and BECA instruments, and an update on each subsystem's technical progress.

(MASS-01.3) Development of Novel Ion Inlet Designs for Laser Desorption Mass Spectrometers that Accommodate Different Surface Sampling Strategies

Adrian Southard¹, Adrian Southard¹, Ricardo Arevalo¹, Friso Van Amerom², Ryan Danell³, Desmond Kaplan³, Julie Llano, Wally Rodriguez⁴, Andrej Grubisic¹, Niko Minasola⁴;
¹University of MD, ²Mini-mass consulting, ³Danell Consulting, ⁴AMU engineering

The recently released 2023-2032 Planetary Science Decadal Survey prioritized several missions that could benefit from an *in situ* high mass resolution analyzer including the Enceladus Orbilander, Ceres and comet surface sample return, and a Venus In Situ Explorer. Recent missions to comets, and Enceladus have revealed that these Solar system

bodies may serve as refuges for prebiotic organic molecules and prospective sites of progressive organic synthesis and/or biological activity. Meanwhile, there is also continued scientific interest in understanding the composition of the bulk silicate Moon particularly across the Moon's South Pole-Aitken basin. The ORDL (OrbitrapTM Research and Development Lab) at NASA GSFC is supporting the development of instruments with an ultrahigh mass resolution Orbitrap mass analyzer ruggedized for spaceflight. These instruments will be able to analyze sample composition, including spatially-resolved correlations between organic and inorganic chemistry, the presence of volatile and refractory organic matter, and its diversity.

Each Orbitrap instrument in development uses laser desorption/ablation ionization to produce ions for analysis, but each must have different ion optics (LDI inlets) to transfer ions successfully into their respective mass analyzers. The CORALS (Characterization of Ocean Residues And Life Signatures) instrument was designed to sample ice residues from Europa held in a metal cup at voltage, while CRATER (Characterization of Regolith And Trace Economic Resources) puts the LDI inlet in direct contact with lunar regolith. Furthermore, AROMA (Advanced Resolution Organic Molecular Analyzer) stores desorbed ions in a heritage linear ion trap prior to injecting them into the Orbitrap, thus, enabling selective ion accumulation and tandem mass spectrometry (MS/MS). In each case, the LDI inlet was designed to accommodate rastering of a UV laser across the sample surface as well as variations in the sample's thickness and conductivity.

Simulations were utilized (SIMION) to trace ion trajectories from the sample surface into the Orbitrap (CORALS/CRATER) or LIT (AROMA) These have guided the optimization of ion transfer efficiency, set voltage supply requirements, and detected the need for steps to mitigate high electric fields.

(MASS-01.4) **Hypervelocity Impact Dissociation in Planetary Mass Spectrometry**
Daniel Austin¹, Brandon Turner, Eric Sevy, Matthew Asplund, Locke Hansen; ¹*Brigham Young University*

Due to the high relative velocity between spacecraft and planetary atmospheres during on-orbit or flyby missions, sampled molecules can dissociate upon impact with analytical instrumentation, resulting in a disconnect between observed and actual species. In addition to dissociation, surface adsorption and subsequent recombination can scramble the observed composition. Unfortunately, little is known about chemical reactions in this regime, and the fate of specific compounds encountered at a given spacecraft velocity is not well characterized. This impact fragmentation is separate from, and independent of the fragmentation due to electron ionization that is also observed in neutral mass spectrometers. We have carried out calculations using transition state theory to predict the critical dissociation velocities for many compounds. These calculations are combined with gas kinetics to characterize the effect for typical closed source geometries and with ion Surface Induced Dissociation data as an experimental analogue. Calculations include larger molecules with astrobiological interest, as well as small molecules typically encountered in planetary atmospheres. We are also developing a device that significantly reduces impact

fragmentation for all compounds by rapidly quenching the highly excited vibrational states of molecules due to impact.

(MASS-01.5) Presentation Title TBD

Stojan Madzunkov¹; ¹NASA

22PAT04: In Situ Spectroscopy for Industrial R&D

Chair: Mark Rickard

(PAT-04.1) In Situ Spectroscopy for Industrial Reaction Monitoring

Xiaoyun (Shawn) Chen¹; ¹Dow

Optical spectroscopy has long been utilized as an effective tool to monitor reactions and process in both academia and industry. There are many potential techniques such as mid-infrared (MIR), near-infrared (NIR), Raman, UV-vis, and fluorescence spectroscopy. Each technique has its own strengths and weakness, and diverse sampling modes. Such a wide range of options makes it challenging to decide on the most effective way to monitor a reaction. In this talk we will share how in situ spectroscopy has been successfully utilized to monitor numerous reactions and the 7 key steps involved: 1. Discuss with process owner and project team whether in situ spectroscopy is the right tool for the task; 2.

Feasibility/proof-of-concept study to choose the appropriate technique; 3. Start with a calibration experiment or a real reaction; 4. Decide on how much data to acquire and how to analyze the data; 5. Validate in situ spectroscopy results; 6. Implement the method and monitor reactions and processes; 7. Think about the potential for implementation in manufacturing.

(PAT-04.2) In Situ IR Study on Polyurethane Reactions

William Wang¹; ¹Lubrizol Advanced Materials

The application of *in situ* IR spectroscopy has been widely used in industry for decades. The method can provide more insightful information on the reaction, which includes the reaction kinetics and possible intermediate(s) involved. Such information drives innovation in process modification and optimization. Lubrizol Advanced Materials is one of the leading manufacturers of thermoplastic polyurethane (TPU) globally. TPUs are engineered polymers that can be tailored chemically to be used in variety of applications. They bridge the gap between rubbers and plastic due to their unique performance, flexibility and melt processability. In this talk, applications of using *in situ* IR spectroscopy to monitor the TPU reaction were discussed, including determining the reaction endpoint by examining the residual isocyanate level; and correction of non-linear response of isocyanate level in prepolymer reaction.

(PAT-04.3) Wide Spectral Range, Large Scanning Area, Cloud Connected and Compact FT-NIR Spectral Sensing Platform for On-site Analysis

Yasser M. Sabry¹; ¹Si-Ware Systems

Diffuse reflectance infrared spectroscopy has gained traction in many industrial applications in recent years due to the emergence of a new generation of low-cost handheld spectrometers that did not exist a decade ago. One of their main applications is in-situ analysis, for instance in-line, at-line, or on-field. This versatility can enable feed millers to maximize the use of high-value raw materials and increase productivity and profitability, instant dairy feed analysis for improved milk production, prediction of soil organic matter and carbon for precision fertilization, authenticity screening in food chains and combating the deadly food-fraud crisis, in addition to many others. However, several challenges exist for on-site, real-time, low-cost and accurate analysis of the samples. In this talk, we shed light on Neospectra based on a chip-scale microelectromechanical system (MEMS)-based FTIR sensing platform. The core engine is highly integrated comprising the self-aligned monolithic silicon MEMS chip, self-aligned micro-optics and a single photodetector in a tiny package enabling the unique compactness of the overall solution. The platform is producible in a scalable way for addressing the hungry market need, handheld, cloud-connected to the chemometric models, and maintains its high-end instrument specification. The wavelength range is 1350 nm to 2500 nm enabling the detection of the first overtones and combination bands and highly sensitive measurements. Moreover, the solution has an innovative optical head capable of measuring inhomogeneous samples taking advantage of the large-area sample coverage by the infrared light. This overcomes the heterogeneity and the pseudo-random spatial arrangement of the grains facing the optical interface, minimizing the prediction errors due to the spectrospatial photometric repeatability. Given the unique advantage of the solution, it is believed that this type of sensing platform will not only revolutionize the analytical technology but also work as a catalyst enabling ubiquitous material analysis.

(PAT-04.4) Monitoring Structural and Chemical Curing Kinetics of Epoxy, Methacrylate, and Dual-Cure Resins for Additive Manufacturing Via In-Situ Raman Spectroscopy

Robert V. Chimenti¹, Alexandra M. Lehman-Chong¹, Jianwei Tu¹, Joeseeph F. Stanzione¹, Samuel E. Lofland¹, James T. Carriere²; ¹Rowan University, ²Coherent Inc.

Developed a novel methodology for in-situ monitoring resin curing kinetics via Low-Frequency Raman spectroscopy.

In resin-based additive manufacturing, the degree of monomer conversion influences the final product's material properties, affecting both printability and fidelity. Vibrational spectroscopy provides a simple and accurate way to measure the extent of cure of the resin. This is traditionally accomplished by monitoring the ratio of intensity of a conversion sensitive band to that of a reference peak post-cure via FTIR-ATR. While effective, this approach only measures conversion at the surface and does not represent the average conversion throughout the bulk and cannot be easily used in-situ. However, since Raman spectroscopy is a non-contact technique, it can be directly integrated into 3D printers for in-situ monitoring of the resin.

In this study, we measured epoxy ($\sim 915\text{ cm}^{-1}$), methacrylate ($\sim 1640\text{ cm}^{-1}$), and dual-cure (epoxy-methacrylate) resins to better understand the curing kinetics. First, we validated this methodology by measuring a fully methacrylate resin system and fitting the results to a photopolymerization kinetic model. Next, we monitored the methacrylate conversion of a dual-cure (epoxy-methacrylate) resin during photopolymerization and analyzed the kinetics profile with the same model. This resin system exhibited a more complex kinetic profile with two distinct regions with unique conversion rates and reaction orders. We hypothesize that a degree of simultaneous epoxy network formation impedes the rate of methacrylate polymerization.

The semi-amorphous structure of polymers leads to a disorder band with a peak around 15 cm^{-1} and a broad torsional band centered around 85 cm^{-1} . Since the disorder band is directly related to the phonon density of states, it is extremely sensitive to polymerization, whereas the torsional band relates to rotational freedom of the side groups, which are far less affected. Utilizing these two bands, we directly evaluated the "structural kinetics" of the resin systems to determine their average bulk kinetic profiles independent of chemical functionality. Not only is this approach chemically agnostic, but we also found that the "structural kinetics" has less noise, higher dynamic range, and provides a strong enough signal to utilize the anti-Stokes measurements, minimizing interference from autofluorescence. These results demonstrate the advantages of using low-frequency Raman to monitor additive manufacturing processes, particularly in large-scale multi-resin 3D printers.

(PAT-04.5) **High Throughput Raman for Low-Volume Crystallization.**

Shamus Driver¹, Mark S. Kemper², Shaun J. Fraser²; ¹*Tornado Spectral Systems*, ²*Tornado Spectral Systems*

This work shows low levels of detection for measuring crystallization in-situ.

Crystallization is a commonly used process for solid-dose manufacturing. To improve the biological performance and economic benefits of a crystallization process, proper process control is necessary. Raman Spectroscopy has been shown to be a ubiquitous process analytical technology ranging in application from the large molecule to the small molecule space. By coupling a Raman spectrometer to a crystallization process, one can observe nucleation, crystallization, and polymorph changes in real-time. This work monitors crystalline polymorph changes in mefenamic acid, carbamazepine, and cannabidiol (CBD). In mefenamic acid, the transition from Form II to Form I was observed. For carbamazepine, the transition from Form III to Form II was observed. With Raman spectroscopy, one can also observe the dissolution of crystals as well as the precipitation. This is the focus of the CBD work. By utilizing a high-throughput Raman spectrometer, spectra can be collected in 5 to 15 seconds, leading to proper process understanding and monitoring.

Recent work using Raman instrumentation integrated with a device for high-throughput, low-volume crystallization development will be highlighted in two of these applications.

22PMA01: Characterization of Therapeutic Modalities: From Small Chiral Molecules to Fibrils and Nucleic Acids

Chair: Rina Dukor

(PMA-01.1) **Presentation Title TBD**

Leo A. Joyce¹; ¹*Arrowhead Pharmaceuticals, Inc.*

(PMA-01.2) **Application of Vibrational Circular Dichroism (VCD) in Drug Discovery and Development – Structure Elucidation of Chiral Molecules**

Yanan He¹, Yanan He¹; ¹*GSK*

Application of Vibrational Circular Dichroism (VCD) in Drug Discovery and Development
– Structure Elucidation of Chiral Molecules

Yanan He

MST-PDS-CMCA-SFC-MS & Characterization

GSK Upper Providence, PA

Determination of absolute configuration (AC) of chiral molecules is important in drug discovery and development of chiral drugs in the pharmaceutical industry. It is critical for understanding structure-property and structure-activity relationship. Vibrational Circular Dichroism (VCD) has become a powerful tool for the determination of the (AC) of chiral molecules in solution state. It offers an alternative or supplement to X-Ray crystallography. By comparing the experimental VCD and IR spectra of an unknown sample with the ab initio density functional theory (DFT) calculated VCD and IR spectra of a chosen configuration, the AC of the unknown sample can be assigned unambiguously. In this presentation the basic principles of the application of VCD to the AC determination of chiral molecules are described. The steps required for VCD measurements and calculations are outlined. Some recent examples of application of VCD in drug discovery and development in GSK are discussed.

(PMA-01.3) **Lilliputian Particles: Scattering and Spectroscopy Applied to New Large Molecule Delivery Vehicles**

Kevin Dahl;

Pharmaceutical drug delivery continues to push the limits of the analytical laboratory to ever smaller bodies to characterize, necessitating new technologies or the revisiting of established techniques. Lipid nanoparticles, viral vectors, virus-like particles, nano-adjuvants, the list grows longer while laboratory scientists struggle to keep up. Optical techniques, including scattering and spectroscopy, provide some of the more modern approaches to characterization and quantification of these wonder carriers. The common tools used in today's characterization laboratories will be reviewed to provide a fresh perspective for these new drug delivery vehicles.

(PMA-01.4) Vibrational Optical Activity to Elucidate the Conformational Behaviour of the Antibiotic Vancomycin and Derivatives

Roy Aerts¹, Wouter Herrebout¹, Christian Johannessen¹; ¹*University of Antwerp*

Vibrational optical activity (VOA) techniques are based on the differential IR absorption or Raman scattering of left and right circular polarized light, respectively referred to as vibrational circular dichroism (VCD) and Raman optical activity (ROA). Besides being of great value in the determination of the absolute configuration of a compound, these techniques are particularly sensitive towards their conformational behaviour. Hitherto, however, mostly biomolecules (purely empirical) and small (model) systems (limited by the computational resources) enjoyed attention. As the computational power increases, fresh, more realistic, and consequently more complex systems can be investigated by means of VOA. To take this next step, the glycopeptide antibiotic vancomycin has been carefully selected. First, a strategy has been developed to reliably determine the conformational ensemble of vancomycin in aqueous solution employing ROA. The performance of ROA herein proves to be surprisingly high. Then, when conducting the same analysis for vancomycin in DMSO using both ROA and VCD, again a satisfactory result was obtained for ROA. VCD, on the contrary, appears to be too sensitive to minor conformational changes, hampering any assignment. In all cases it was found that the carbohydrate entity barely contributes to the overall VOA spectrum of vancomycin. These observations were put to the test by the recording and analysis of VOA spectra of three derivatives: oritavancin, dalbavancin and teicoplanin. Finally, vancomycin is being studied when bound to its biological target, Lipid II, by using ROA. All combined, the obtained results provide important insights in the applicability of the VOA techniques in the conformational elucidation of challenging, real-world molecular systems.

(PMA-01.5) Lipids Reverse Supramolecular Chirality and Reduce Toxicity of Amyloid Fibrils

Kimberly Quinn¹, Stanislav Rizevsky², Kiryl Zhaliyazka², Mikhail Matveyenka², Dmitry Kurouski²; ¹*BioTools*, ²*Texas A&M University*

Abrupt aggregation of misfolded proteins is a hallmark of many medical pathologies including diabetes type 2, Alzheimer and Parkinson diseases. This results in the formation of amyloid fibrils, protein aggregates with distinct supramolecular chirality. A growing body of evidence suggests that lipids can alter rates of protein aggregation. In this study, we investigated whether lipids could alter the supramolecular chirality of amyloid fibrils. We found that if present at the stage of protein aggregation, phospho- and sphingolipids uniquely reversed supramolecular chirality of insulin and lysozyme fibrils. Furthermore, amyloid fibrils with opposite supramolecular chirality exerted distinctly different cell toxicity. Specifically, insulin and lysozyme fibrils with reversed supramolecular chirality were less toxic to cells than the aggregates with normal supramolecular chirality. These findings point on the important role of lipids and supramolecular chirality of amyloid fibrils in the onset and progression of amyloid diseases.

22RAM01: Emerging Raman

Chair: Pavel Matousek

(RAM-01.1) Wearable/Flexible Surface-Enhanced Raman Spectroscopy

Keisuke Goda¹; ¹*The University of Tokyo*

The last two decades have witnessed a dramatic growth of wearable sensor technology, mainly represented by flexible, stretchable, on-skin electronic sensors that provide rich information of the wearer's health conditions and surroundings. However, most wearable sensors are based on measurements of physical parameters, not chemical. In this talk, I introduce a highly scalable, wearable surface-enhanced Raman spectroscopy (SERS) substrate based on an easy-to-fabricate, low-cost, ultrathin, flexible, stretchable, adhesive, and bio-integratable gold nanomesh. It can be fabricated in any shape and worn on virtually any surface for label-free, large-scale, in-situ sensing of diverse analytes from low to high concentrations (down to 10 nM). To show the practical utility of the wearable SERS sensor, I show its application to the detection of sweat biomarkers, drugs of abuse, and microplastics. This wearable SERS sensor represents a significant step toward the generalizability and practicality of wearable sensing technology.

(RAM-01.2) Computational Stimulated Raman Scattering Microscopy

Ji-Xin Cheng¹, Ji-Xin Cheng¹; ¹*Boston University*

Providing molecular fingerprint vibration information and high imaging speed, coherent Raman scattering microscopy, based on either coherent anti-Stokes Raman scattering (CARS) or stimulated Raman scattering (SRS), allows real-time vibrational imaging of living cells and/or tissues with sub-micron spatial resolution. These instrumentation-based advances, however, do not fulfill all the desired parameters in hyperspectral imaging, including broad bandwidth, high signal to noise ratio (SNR) and high speed. In pushing these physical limits, it is common that one parameter is optimized at the price of sacrificing other advantages. We have developed two complementary platforms that will allow high-speed, high-content, and high-sensitivity mapping of cell metabolism. The first platform is for samples without prior knowledge. We will build a polygon scanner to tune the delay between two chirped pulses on a 20-microsecond time scale. We will then deploy deep spatial-spectral learning to denoise the low-SNR hyperspectral measurements and extract salient information with much enhanced SNR. This integrated approach effectively bypasses the conventional tradeoff between acquisition speed and SNR and enables high-speed, high-throughput, hyperspectral SRS imaging using informative fingerprint Raman bands. The second platform is for samples with known target species. We will develop a sparsely sampled hyperspectral imaging strategy to increase the overall speed by one order of magnitude while maintaining the same SNR. We will develop a novel approach to determine the minimum number of essential frames. On the instrumentation side, a fast-tuning fiber laser will be deployed to acquire a sparsely sampled hyperspectral stack within one second for the study of living systems.

(RAM-01.3) Investigating the Antimicrobial Properties of the Peptide, LL-37, in Preventing E. Coli Biofilm Forming: a Raman Microscopy-Based Approach

Samantha L. Walker¹, William J. Tipping¹, Yun Xu², Sian Sloan-Dennison¹, Royston Goodacre², Howbeer Muhamadali², Duncan Graham¹, Donald Davidson³, Karen Faulds¹;
¹The University of Strathclyde, ²The University of Liverpool, ³The University of Edinburgh

Society greatly relies on antibiotics, enabling surgeries and treatments such as organ transplants and dialysis. However, World Health Organisation (WHO) predicted antimicrobial resistance (AMR) could cause 10 million deaths by 2050 if nothing is done. Biofilms are another source of antibiotic failure and cause up to 80 % of all infections. They form by bacteria attaching to a surface and forming microcolonies in a matrix of polysaccharides, proteins and DNA that connect through water channels. Once mature, cells disperse from the microcolonies, completing the life cycle. Their resistance (10- to 1000-fold increase) is unclear, although the matrix and microenvironments may offer protection. Some antimicrobials retard biofilm growth at sub-killing concentrations, but the mechanisms are unknown. Antimicrobial peptides (AMPs) are conserved within the immune system of all organisms and were recently shown to reduce and distort biofilm growth at sub-killing concentrations. LL-37, known for modulating the human immune system and as an antimicrobial, is an excellent antibiofilm agent but how this is achieved is unclear. Analysis is typically limited to destructive techniques, such as dried samples via atomic force microscopy, or using chemical stains combined with fluorescence microscopy and ultraviolet spectroscopy. Non-destructive, label-free techniques are required to understand formation and antimicrobial response to guide future strategies against AMR. This work focused on monitoring *Escherichia coli* biofilm formation over time using Raman spectroscopy coupled with chemometrics, principal component analysis and partial least squares regression. Key peak changes were clearly visible through the different stages of the biofilm development, with the largest difference seen after 4 h. Compared against the classic staining method, crystal violet, this approach offered easier sample preparation while providing detailed biochemical information on structural and metabolic changes. Moreover, stimulated Raman spectroscopy rapidly inspected biofilm structure, which displayed key changes from the initial attachment through to the dispersal step using wavelengths 2930 cm⁻¹ (protein), 2975 cm⁻¹ (DNA) and 2850 cm⁻¹ (lipid). The success of these Raman-based approaches to detect specific chemical and structural changes in a live biofilm with minimal sample preparation will be applied to elucidate the antibiofilm properties of LL-37, comparing it against approved antibiotics.

(RAM-01.4) Development of A Multifocal Spot Raman Spectrophotometer for High-Throughput Biological and Chemical Screening using 96 Microplates

Hao-Xiang Liao¹, Kazuki Bando¹, Menglu Li¹, Katsumasa Fujita¹; ¹Osaka University

Developed composition discrimination method with Raman scattering for high-throughput screening using 96 microplate.

In these two decades, high-throughput screening has been a prominent method in the field of biochemical and pharmaceutical research because it can screen large numbers of samples in a short time. Presently, in high-throughput screening, fluorescence-based assays are the mainstream for chemical composition analysis. Although fluorescent labeling is powerful, it

has the limitation that measurements are limited to only those objects that can be stained. In addition, fluorescently labeled specimens may be difficult to use for pharmacological testing or medical treatment after screening.

Raman spectroscopy has the potential to bring a capability of label-free chemical analysis into high-throughput screening because it can provide vibrational information of a sample to discriminate targets in a label-free manner and monitor the change of analytes. However, Raman spectroscopy is not widespread in the field of high-throughput screening because the small Raman scattering cross-section requires orders-of-magnitude longer measurement time compared to fluorescence. To measure analytes isolated in the wells of a microplate, the conventional Raman system needs to measure the spectrum well-by-well, with each measurement taking a few seconds to minutes. As consequence, it is not possible to monitor all the analytes simultaneously.

Here we presented a Raman spectrophotometer that can measure Raman spectra from the samples loaded in microplates simultaneously. It equips with 96 lens arrays for multiple-excitation and fiber-based multiple-collection which makes it possible to use a sensitive 2D camera to measure the Raman spectra from 96 analytes in single laser exposure. The system has the feasibility to detect and monitor the pharmaceutical and biochemical reaction, for example, discriminating crystal polymorph and observing crystallization kinetics; or the drug response of cells or proteins.

(RAM-01.5) Efficient Separation and Characterization of Biomolecules by Optical Tweezers-Controlled Surface-Enhanced Raman Spectroscopy

Jinqing Huang¹, Xin Dai¹, Wenhao Fu¹, Vince St Mesias¹, Wei Liu¹, Jinqing Huang¹; ¹*The Hong Kong University of Science and Technology*

Surface-enhanced Raman spectroscopy (SERS) empowers ultra-sensitive detections of chemicals and biological analytes in dilute solutions, owing to the enhanced electric field provided by the metallic nanostructures as SERS substrates. An efficient way to generate and control SERS-active nanostructures would be desirable to improve the reproducibility, stability, and bio-compatibility of existing SERS analytical methods. Here, we integrate optical tweezer manipulations and SERS measurements to create dynamic plasmonic hotspots and conduct in-situ SERS characterizations of various analytes in aqueous solutions. Without the addition of aggregating agents, it provides spatial and temporal control to form the SERS-active assembly from the dilute silver nanoparticle colloid in as low as nM-level concentrations, which minimizes the influence on the detection system. Moreover, it enables the visualization and manipulation of the hotspot between two silver nanoparticle-coated silica beads to generate tunable and reproducible SERS enhancements with single-molecule level sensitivity. When connected to a microfluidic system, this dynamic SERS detection window characterizes the secondary structures of the passing-by proteins without perturbation to their native states. Under high-throughput screening, it helps to identify the transient species of alpha-synuclein (an intrinsically disordered protein closely linked to Parkinson's disease) to reveal the mechanism of amyloid protein

aggregation. Furthermore, the integrated optical tweezers provide additional control on the analytes ranging from small molecules to large cells for efficient collections. Hence, this optical tweezers-controlled surface-enhanced Raman spectroscopy platform holds great potential for various bio-applications, especially the detection and separation of low-populated biomolecules (DNA, RNA, and protein) under physiological and *in vivo* conditions.

22RAM02: SERS 1

Chair: Royston Goodacre

Co-Chair: Sian Sloan-Dennison

Co-Chair: Zac Schultz

(RAM-02.1) Automated Nanoparticle Synthesis for Improved SERS-based Sensing
Samuel Mabbott¹, Samuel Mabbott¹; ¹*Texas A&M University*

Arriving in the laboratory after a COVID instigated hiatus, the students and I were excited to rejuvenate our postponed research developing SERS-based sensors. Unfortunately, despite our best efforts to preserve our transduction agents and assay components before departing the lab, very few of our sensors matched the performance we had witnessed pre-lockdown. The decrease in analytical performance initiated our group to review how we should best proceed with developing our sensors in the future. A common theme within our research is using gold nanoparticles (AuNPs) to enable SERS-based sensing. While we use a wide morphological array of AuNPs in our platforms, the most common particles we use are nanospheres synthesized by manual solution-based methodologies. Knowing that there could be some reproducibility issues associated with the fabrication of the nanospheres, we conducted a small study whereby researchers in the laboratory were tasked with synthesizing 30 nm AuNS using a citrate reduction protocol. Upon evaluation of the results, variation in the nanoparticle size and the respective SERS signal generated was too large to sustain continued sensor development. To overcome the reproducibility issue, we have adopted an automated synthesis approach to provide long-term support for nanoparticle fabrication throughout sensor development and application. In my talk, I will share with you details of our automated system, the benefits of its usage, and discuss an alternative small form factor system that could be easily integrated into the workflow of a research laboratory.

(RAM-02.2) Development of SERS-Based Assay Platforms for Rapid and Accurate Diagnosis of SARS-CoV-2

Jaebum Choo¹, Jaebum Choo¹; ¹*Chung-Ang University*

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has caused significant social and economic problems worldwide. Currently, RT-PCR, which detects RNA inside a virus, is used as the standard diagnostic method for SARS-CoV-2, but the total diagnostic time, including sample preparation, gene amplification, and detection, requires approximately 3-4 h. Various rapid kits for immunodiagnosis using antigen-antibody reactions have also been developed and commercialized to shorten the diagnosis time. However, they have not been adopted as the standard diagnostic method owing to

their low limit of detection and poor accuracy. In particular, a false-negative result obtained by the commercialized immunodiagnostic kit is a severe problem that can aggravate the spread of SARS-CoV-2. To resolve this problem, we developed new SERS-based various assay platforms to quantify SARS-CoV-2 lysates with high sensitivity. This presentation will introduce three different types of SERS-based assay platforms; a SERS-based lateral flow assay (LFA) immunodiagnostic strip with a portable Raman reader, a SERS aptasensor using nano-popcorn substrates and an Au-Nanoparticle-Internalized nano-dimple SERS-PCR sensor. Our SERS-based assay platforms show a strong potential to resolve the problems in terms of low sensitivity and limit in quantitative analysis inherent in conventional antigen tests to detect SARS-CoV-2. The results of this study demonstrate the possibility of a clinical application that can dramatically improve the detection limit and accuracy of the currently commercialized SARS-CoV-2 immunodiagnostic kit.

(RAM-02.3) Into Another Dimension: Coupling Multidimensional Chromatography and SERS

Christa Brosseau¹, Maddison M. Eisnor¹; ¹*Saint Mary's University*

In this talk, we will discuss our recent work at the interface of multidimensional chromatography (2D-LC) and surface-enhanced Raman spectroscopy (SERS). In particular, we have been exploring electrochemical SERS (EC-SERS) as an offline detection modality for the dilute fractions which are collected from the second dimension of the 2D-LC. As a proof-of-concept system, we have been exploring green tea as a model of a complex mixture. Once successful, we then moved on to an even more challenging complex sample, dissolved organic matter (DOM) collected from a local river. In this presentation we will highlight the power of combining a powerful multidimensional separation with a sensitive and selective detection platform like EC-SERS. As part of this talk we will discuss the challenges and opportunities in combining multidimensional chromatography and SERS.

(RAM-02.4) Differentiation of Glycans by Surface Enhanced Raman Spectroscopy

Hannah C. Schorr¹, Zac D. Schultz¹; ¹*The Ohio State University*

Surface enhanced Raman spectroscopy and principal component analysis were used to differentiate between various glycans.

Glycosylation, a common post-translational modification to proteins, can impact the interactions and functions of a protein. Glycoproteins are becoming commonly identified as drug delivery targets and are useful as therapeutics. To better understand glycoprotein interactions and makeup, it is necessary to find a rapid and easily accessible method for characterizing the glycome. Analyzing the glycome with common techniques such as mass spectrometry is difficult, however, due to the similar molecular weights and isomeric structures of many glycans. Surface enhanced Raman spectroscopy (SERS) provides a solution to this challenge as it probes the vibrational modes of bonds within a molecule. Sugars are also difficult to detect in SERS; however, here we demonstrate that by conjugating sugars to a boronic acid, a unique SERS response can be generated for each glycan. Based on changes in the SERS spectrum, principal component analysis (PCA) can be used to create a model that separates the sugars by type. The PCA model can then be

used to identify unknown monosaccharides based on their SERS spectrum. This approach appears useful both when the sugars are fixed to a substrate via a thiol bond as well as when they are freely flowing over a SERS substrate, thus allowing for further applications in tandem with liquid chromatography separation and extraction from a complex matrix.

(RAM-02.5) Differentiation of Structurally Similar Fentanyl Analogs with Theoretical and Experimental Analysis by Surface-Enhanced Raman Spectroscopy (SERS)

Sevde Dogruer Erkok¹, Emily Hernandez¹, Bruce McCord¹; ¹*Florida International University*

The results can be implemented in field where portable-Raman spectrometers are used in drug analysis.

New synthetic opioids, especially fentanyl and its analogs, are causing the most recent acceleration in opioid abuse. The presence of fentanyl analogs as mixtures in illicit drugs makes it hard to estimate their potencies. This makes the detection and differentiation of fentanyl analogs critically significant. Most of the screening methods in current use have difficulty in detecting the full range of opioid analogs due to a wide variety of structural variations. However, Raman spectroscopy, specifically surface-enhanced Raman spectroscopy (SERS) is quite capable of detecting and identifying previously known and/or unknown fentanyl analogs. The SERS technique uses Raman spectroscopy combined with colloidal metal nanoparticles to yield highly sensitive SERS spectra. It can also differentiate structurally similar fentanyl analogs due to its ability to yield spectroscopic fingerprints for the detected molecules. Certain fentanyl analogs such as carfentanil, furanyl fentanyl, acetyl fentanyl, 4- fluoroisobutyryl fentanyl, and cyclopropyl fentanyl, have gained popularity and constitute 76.4 percent of the fentanyl analogs identified in drug seizures. Several of these have been already described using Raman spectroscopy. However, there are many other fentanyl analogs that are structurally similar to 4-fluoroisobutyryl fentanyl or cyclopropyl fentanyl. Thus, it is important to differentiate these analogs from similar molecules in order to track and identify trends in illicit distribution. In this presentation, we develop methods for the differentiation of structurally similar fentanyl analogs using theoretical and experimental methods. To do this, a set of fentanyl analogs were examined using Density Functional Theory (DFT) calculations. These results were then compared with Normal Raman and SERS techniques and analyzed using statistical methods. Structurally similar fentanyl analogs have been able to be differentiated from each other. Structurally similar fentanyl analogs have been able to be differentiated from each other. The ultimate goal of the project will be to assist law enforcement in identifying and differentiating unknown fentanyl analogs individually and in drug mixtures. The experimental results obtained in this project can be readily implemented in field applications and in the smaller laboratories, where inexpensive portable Raman spectrometers are often present and are used in drug analysis.

22RAM16: Methods for Real Samples

Chair: Robert Lascola

(RAM-16.1) Rapid Analysis Of Refined Fuel Properties Using A Novel Solid-State Raman Analyzer

Thomas Dearing¹, John Richmond¹; ¹*MarqMetrix, Inc.*

Allows technicians to analyze multiple fuel properties in less than 5 seconds

Raman spectroscopy is an ideal candidate for refined fuels analysis, having the key attributes of specificity, sensitivity, speed and stability and simplicity. In this paper, we will describe the methodology for collecting high quality Raman spectra and the strategy for turning the spectral data into information to inform the decision making process. Analysis of multiple fuel types including gasoline, jet and diesel as well as component streams such as reformat, isomerate and alkylate will be described as well as modeling strategies to ensure a simplified approach for laboratory workflow for technicians and operators

(RAM-16.2) New Innovative Raman Sampling Techniques Enable Quantitative Measurements on Raman Microscopes.

Harry Owen¹; ¹*HORC*

Raman microscopes will be able to do quantitative measurements on bulk heterogenous solid samples

This presentation will discuss three new Raman sampling techniques that when combined enable a Raman microscope is to do sensitive and robust quantitative analysis of bulk heterogeneous samples e.g., pharma tablets

1. “Collimated Laser with Backscatter Collection” for Representative Sampling”

Diffuse scattering and photon migration within the sample enhance the Raman signal and generate representative sampling.

The collimated not focused laser output beam from the microscope significantly reduces unwanted Raman signals from surface coatings making it ideal for coated tablets and capsules.

2. “Real-Time Raman Calibration” for Improved Spectral Precision.

Unwanted wavenumber shifts observed in Raman spectra from the laser and/or spectrograph due to thermal drifting or diode instability can be corrected for by using the Raman reference band at 321.0 cm⁻¹ from an integrated CaF₂ optic superimposed onto each collected Raman spectra.

3. “Gold-Plated Sample Holder” for Extended Representative Sampling and Raman Signal Amplification.

By locating the solid sample within a gold-plated sample holder based on an integrating sphere design the gold reflectivity (>90%) over the complete wavenumber range significantly extends the laser pathlength enhancing both the Raman signal and the sampling volume including the outside parts of the sample.

The design of the new type of microscope objective and gold-plated sample holder will be described, and Raman spectra collected from a range of samples demonstrating the benefits of these new techniques will be presented.

(RAM-16.3) In-Process Laser Based Method for Detection Impurities at Trace Levels
Edward A. Orr¹; ¹*ABB Inc.*

Ultra-Low level, Rapid Detection of Targeted Impurities Enables Process Engineers to Implement Effective process Control

Off-Axis Cavity Output Spectrometers (OA-ICOS) combines exceptionally long pathlengths with the a scanning laser, The ability to collect a spectrum permits the usage of algorithms to minimize the impact of interfering spectral features. These measurements can be made in 10 seconds, permitting effective process control for asset protection. Examples of process results for three types of process will be presented along with an overview of the analyzer design.

The importance of ultra-low level, very rapid analysis is a key feature for asset protection, comparing this technology to more traditional methods will be giving with comparative data.

(RAM-16.4) Development of New Raman Gas Schemes with High Isotopic Discrimination and for the Analysis of Volatile Organic Compounds

Torsten Frosch¹, Andreas Merian², Timea Frosch², Jürgen Popp³; ¹*Technical University Darmstadt*, ²*Leibniz Institute of Photonic Technology*, ³*Leibniz Institute of Photonics Technology*

Fiber-enhanced-Raman-spectroscopic analysis of stable gas-isotopes (^{12/13}CO₂ and ^{16/18}O₂) and VOCs for environmental sensing.

Raman gas spectroscopy provides high chemical selectivity for the analysis of multiple gases in complex mixtures [1-5]. We developed a gas sensing scheme based on micro-structured optical hollow core fibers to improve the sensitivity low ppm concentrations.

In doing so we were able to analyze complex fuel gas mixtures and detect major components (methane) alongside minor fuel gas components (C2-C6 alkanes) and hydrogen in one measurement [1]. In a second study we advanced the abilities to quantify volatile organic compound (methanol) simultaneously with methane, hydrogen, and carbon dioxide to study the methylotrophic pathway microbial methanogenesis [3], which is a key biogeochemical process in the carbon cycle that is responsible for 70% of global emissions of the potent

greenhouse gas methane. Finally, we addressed the very good isotopic discrimination of the technique and demonstrated combined $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ as well as $^{18}\text{O}_2$ and $^{16}\text{O}_2$ measurements [4]. This stable isotope tracing has powerful applications in ecology to track and disentangle different processes and pathways [4]. Especially for studies focused on the gas exchange of plants, sensing techniques are sought-after that offer oxygen (O_2) and carbon dioxide (CO_2) sensitivity with isotopic discrimination [4].

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(RAM-16.5) Capability of Portable Shifted Excitation Raman Difference Spectroscopy for Real-World Investigations

Martin Maiwald¹, Kay Sowoidnich¹, André Müller¹, Bernd Sumpf¹; ¹*Ferdinand-Braun-Institut*

SERDS transfers Raman spectroscopy out of the laboratory and is successfully demonstrated in real-world applications

Shifted excitation Raman difference spectroscopy (SERDS) has been applied successfully for various studies under laboratory conditions. However, outdoor investigations are becoming increasingly important for on-site sampling and this, in turn, increases the demand for portable SERDS instruments. Applying two slightly shifted excitation wavelengths, SERDS can efficiently reject disturbing background signals such as fluorescence and ambient lights and is therefore particularly beneficial for applications outside usual laboratory environments.

In this contribution, we present portable SERDS as a powerful tool for selected real-world investigations. To perform on-site SERDS experiments, a portable and robust sensor system is realized. Dual-wavelength diode laser emitting at 785 nm are developed as the excitation light source for Raman spectroscopy and SERDS. These devices provide two excitation lines with a flexible spectral distance up to 30 cm^{-1} at an optical output power up to 150 mW. A

stable spectral behavior of both laser lines from continuous wave operation to a rapid alternating operation is achieved and provides an exposure time down to 25 ms for each excitation wavelength using the realized portable SERDS sensor system. This enables real-time SERDS investigations which are required for rapidly changing measurement conditions such as fluorescence quenching and for application fields which need quick on-site decisions.

To demonstrate the capability of SERDS in real-world settings, portable SERDS sensor systems are used in agriculture applications. Here, apples and green apple leaves are investigated directly on the trees. Moreover, the potential of portable SERDS is evaluated for the analysis of complex and highly heterogeneous soils and on-site investigations were carried out on an agricultural test plot containing selected soil types. The measurements have shown that SERDS can efficiently extract Raman signals from laser-induced fluorescence and daylight contributions and generates a 15-fold improvement of the signal-to-background-noise ratio. This enables the identification of characteristic Raman signals of selected target substances such as chlorophyll or soil components.

These results demonstrate the successful applicability of portable SERDS sensor systems as a powerful tool for real-world investigations outside common laboratory environments. Beside precision agriculture our study furthermore shows the potential for additional application areas, e.g., geology and cultural heritage.

22SPECIAL01: Coherent Multidimensional Spectroscopy Symposium I

Chair: Wei Zhao

(SPEC-01.1) Multidimensional Floquet State Spectroscopy and Its Applications to Analytical Chemistry and Coherent Control of Reactions

John C. Wright¹, John C. Wright¹, Roger Carlson, Wei Zhao², Mark Rickard³, Nathan Mathew, Lena Yurs, Erin Boyle, Peter C. Chen⁴, Daniel Kohler, Kent J. Meyer⁵, Jonathan Handali, Emily Kaufman, Kyle Sunden; ¹University Wisconsin-Madison, ²University of Arkansas at Little Rock, ³DuPont, ⁴Spelman College, ⁵UW Madison

Floquet state spectroscopy is the first fully coherent optical analogue of multiple quantum coherence nuclear magnetic resonance (NMR) and like NMR, is ideal for characterizing complex samples. It is based on creating a multiple quantum coherence of vibrational and/or electronic states using tunable excitation pulses. Scanning the pulse frequency across vibrational and/or electronic resonances creates multidimensional spectra with cross-peaks that directly measure the coupling between states. Although this methodology is ideal for analytical and materials chemistry applications, its use has been sharply constrained by the lack of commercial laser systems capable of continuous wavelength scanning across wide spectral ranges while maintaining spatial and temporal alignment. In collaboration with Light Conversion, we have developed a laser system that creates 3 tunable pulses and maintains alignment over frequency ranges from 18 μ m-310 nm. This system can create 3D spectra and 3D spectral fingerprints for label-free microscopic imaging. Scanning time delays between pulses allows measurement of quantum state dynamics.

One of the special characteristics of a Floquet state is that its individual states are not the same as those defined when the system is in its ground state. The Floquet state is a quantum mechanically entangled state where all states are present simultaneously. It therefore allows for coherent control of chemical reactions. Since reactions result from thermally driven bending, twisting, stretching, etc. motions that change the electronic bonding, Floquet states can be created that duplicate these motions but now with complete coherent control. This talk will provide experimental examples of coherent control of reaction dynamics.

(SPEC-01.2) Time Resolved Nonlinear Spectroscopy of Excess Electrons in Aliphatic Ionic Liquids

David A. Blank¹, Andrew T. Healy¹; ¹*University of Minnesota*

Applications of Ionic liquids have become common in radiolytic environments such as solar cells and nuclear reactors. The photodetachment of an electron from an ion in the liquid creates an initially delocalized and highly reactive species that subsequently localizes to a cavity in less than a picosecond. This is followed by relaxation and cooling that spans decades in time. We apply pump-probe spectroscopy to quantify the reactivity and localization time scales. In order to gain more detailed insight into the structure of the excess electron we apply resonance enhanced femtosecond stimulated Raman spectroscopy (FSRS) to probe the structure and dynamics following photo-detachment in aliphatic ionic liquids. The results will be discussed in the context of the molecular structure of solvated electrons in these liquids and how that structure evolves during localization and solvation. The figure provides an example of time-resolved resonance Raman spectroscopy of the electrons following photodetachment at 266 nm from a pyrrolidinium dicyanamide ionic liquid.

(SPEC-01.3) High Resolution 2DIR spectroscopy

Peter C. Chen¹, DeAunna Daniels¹, Thresa Wells²; ¹*Spelman College*, ²*Georgia State University*

2DIR is a well-established technique that reveals and makes use of the relationship between peaks in vibrational spectra. This talk describes the development of a new high resolution 2DIR instrument that can be used to resolve rovibrational peaks of gas phase molecules.

(SPEC-01.5) Hyperspectral Chemical Imaging with Sum-Frequency Generation Microscopy

Nien-Hui Ge¹, Nien-Hui Ge¹, Hiroaki Maekawa¹, S. K. Karthick Kumar¹, Sudipta Mukherjee¹; ¹*University of California at Irvine*

Chemical imaging with fast acquisition of phase-resolved, polarization-selective vibrational spectra is essential for providing molecular level understanding of many chemical, biological, and materials systems. In this talk, we will report our recent development of a vibrationally resonant sum-frequency generation microscope capable of hyperspectral imaging with high phase stability. The microscope utilizes femtosecond pulses with an 80-MHz repetition rate. It can achieve submicrometer lateral resolution with multimodal

compatibility. It enables multiplex detection in the frequency domain at each sample location by using a broadband mid-IR light to cover a wide spectral range of interest. This advantage overcomes experimental difficulties associated with frequency tuning using a narrow band laser system. Moreover, we have achieved phase retrieval of sum-frequency signal by heterodyne detection in the same microscope setup with self-phase-stabilized spectral interferometry. Applications to visualize C–H stretching modes in collagen tissues of rat tail tendons and potato starch granules have been demonstrated. Within the same amount of data acquisition time that our previous microscope can measure one phase-resolved image at a single wavelength, our new microscope can measure such images at 400 different wavelengths with superior phase stability. Wavelength dependent phase-resolved images of collagen suggest that the measured phase depends on the orientation and tilt angle of triple-helix, and which modes are being probed. The multiplex and heterodyne detection capabilities make vibrationally resonant sum-frequency generation microscopy an attractive addition to the family of hyperspectral imaging techniques.

22SPECIAL01: Coherent Multidimensional Spectroscopy Symposium II

(SPEC-01.4) Opportunities for Ultrafast 2D-IR Spectroscopy in Zeolite Catalysis Research

Paul Donaldson¹, Russell Howe, Alex Hawkins, Gregory Greetham¹; ¹*STFC Central Laser Facility*

Understanding chemical transformations in solid acid Zeolite catalysts has been a fundamental area of catalysis research for many decades. Impacting on issues such as energy, sustainability, pollution control and industrial scale feedstock generation, an incredible amount of knowledge has developed globally regarding Zeolite preparation, characterization and chemistry. Infrared spectroscopy plays a fundamental role in Zeolite research and is well suited to in-operando monitoring of the evolution of the great diversity of hydroxyl, hydrocarbon and framework species present in Zeolite systems. As an IR-heavy chemical research area, Zeolites are ripe for 2D-IR exploration, but as Zeolites are optically scattering powders of micron-sized crystals, they present practical challenges for the acquisition of 2D-IR data under conditions suitable for catalysis studies.

In this contribution, we report on 2D-IR spectroscopy studies of pressed pellets of Zeolites and other microcrystalline catalysts under variable gas flow and temperature. We will discuss an experimental approach that supports routine collection of high quality 2D-IR spectra in the presence of intense pump scattering in both parallel () and perpendicular () pump-probe polarizations. Using this approach, we have explored in-depth the 2D-IR spectroscopy of a range of Zeolites in gas flows of D₂O, HOD/H₂O and MeOD over wide temperature ranges (20C-550C). From this ‘encyclopedia’ of spectra gathered, we will present several observations and procedures for which the uniqueness of Zeolite 2D-IR spectroscopy delivers high value information of importance to contemporary catalysis research.

22SPR01: Emerging Plasmonic Materials and Architectures

Chair: Jean-Francois Masson

(SPR-01.1) Chemical Design of Colloidal Copper-Based Plasmonic Nanocrystals
Xingchen Ye¹; ¹*University of Indiana Bloomington*

In this talk, I will discuss our recent progress on the development of heterometallic seed-mediated synthesis methods for monodisperse shape-controlled copper-based nanocrystals. Using Au nanocrystals as seeds, uniform penta-twinned copper nanorods can be synthesized with tunable aspect ratios ranging from 2 up to 15. Recently, we have also developed strategies for copper-based dilute alloy nanocrystals of diverse shapes including triangular prisms, tetrahedra and octahedra. Correlative single-particle darkfield and ensemble extinction spectroscopic measurements elucidate the shape-dependent plasmonic properties including tunable dipolar and strongly coupled dipolar and quadropolar plasmonic resonances. These facet-defined copper-based nanocrystals are finding new applications in plasmonics, electrocatalysis and photocatalysis.

(SPR-01.2) Ultrabright Nanorattle Assay for Multiplexed SERS Detection of Molecular Biomarkers in Head and Neck Squamous Cell Carcinoma

Joy Q. Li¹, Julia Canick¹, Hoan Ngo², Priya Dukes¹, Walter Lee¹, Tuan Vo-Dinh¹; ¹*Duke University School of Medicine*, ²*John's Hopkins University*

Plasmonic, multiplexed detection of mRNA biomarkers for HNC in unamplified RNA extract.

Head and neck squamous cell carcinoma (HNSCC) is a particularly morbid and fatal disease; the rapid diagnosis and treatment initiation of HNSCC are necessary to give patients a chance at cure. A lack of access to resources and personnel in low-to-middle income countries (LMICs) contributes to a high barrier to patient access to care, increasing the mortality rate of HNSCC. Molecular biomarkers provide a promising diagnostic method, though this usually requires access to a standard laboratory. To address this resource gap in LMICs, we developed a point-of-care, plasmonic assay for the direct molecular diagnosis of HNSCC. The assay utilizes surface-enhanced Raman scattering (SERS)-active metallic nanoparticles called “nanorattles.” These nanorattles are bimetallic with a core-gap-shell architecture; the gap can be filled with SERS-active dyes that produce very high SERS signal. Due to the sharp, unique peaks of characteristic Raman spectra of different dyes, SERS techniques are highly suitable for multiplexed detection. Here, we demonstrate the unamplified and multiplexed detection of HNSCC-associated mRNAs in various HNSCC and non-HNSCC cell lines.

(SPR-01.3) Analysis of Nanostar Reshaping Kinetics for Optimal Substrate Fabrication

Der Vang¹, Pietro Strobbia¹; ¹*University of Cincinnati*

Understanding the gold nanostars reshaping kinetics can help predict and aim for an optimal LSPR.

Gold nanostars (NS) are emerging as an ideal nanoparticle type for surface-enhanced Raman scattering (SERS) applications because of their wide localized surface plasmon resonance (LSPR) tunability, simple synthesis procedure, and high SERS enhancement. These particles are commonly used with a stabilizing coating shell (e.g., thiolated molecules or silver shell). However, coatings cannot be used for the fabrication of SERS substrates as the NS have to interact with the substrate planar surface. Without stabilizing coating, NS have been observed to change over time, leading to a hypochromic shift of the LSPR. We synthesized surfactant-free gold NS with different branching densities and investigated their reshaping morphology and kinetics to understand this shift. Using TEM, the NS' sharp spike features were observed to reshape over time. The kinetics of this process were analyzed and determined by monitoring LSPR over time. LSPR changes follow an exponential decay over time. We were able to fit the data as a function of time and use the initial LSPR (independently of the branch density) to predict the LSPR at a specific time. We show the LSPR's effect on the SERS signal for the NS and how the SERS signal correlated to our prediction. Finally, we tested our approach by making substrates with immobilized NS and collecting the reflectance spectra. We were able to predict and aim for an optimal LSPR with low percent error. These new insights on NS reshaping can permit the fabrication of NS-Based substrates with desirable optical/plasmonic properties.

(SPR-01.4) A Plasmonic Puzzle: The Curious Properties of Hollow Metallic Nanoshells Prepared by the Galvanic Replacement of Silver

Gregory Wallace¹, Ewen Smith¹, Tell Tuttle¹, Karen Faulds¹, Duncan Graham¹; ¹*The University of Strathclyde*

Developing an understanding of why the optical properties of hollow metallic nanoparticles change under irradiation.

Metallic nanoshells exhibit unique optical properties related to the presence of a thin metallic shell surrounding an inner template. These templates include dielectric, polymeric, and metallic nano- and microparticles. By tuning the inner and outer dimensions of the nanoshells, it is possible to readily tune their optical properties from the visible through the infrared regions. One specific property of interest is their photothermal capabilities, as they are far superior to that of classical plasmonic nanospheres. Coupling this effect with plasmon-enhanced sensing techniques, such as surface-enhanced Raman scattering (SERS), has allowed for metallic nanoshells to emerge as an exciting structure for applications in theranostics.

Hollow metallic nanoshells are typically prepared using a sacrificial template that is removed either during or after the growth of the nanoshell. For example, an inner silica core can be removed by exposing the nanoshells to hydrofluoric acid. Alternatively, galvanic replacement of an initial metallic nanoparticle can be performed. Here, cobalt nanoparticles are a quintessential example, however, the synthesis of cobalt nanoparticles requires the use of an inert atmosphere. With a desire to work under ambient atmospheric conditions, we instead use silver nanoparticles as a template for our galvanic replacement. Interestingly, we observed

a series of new optical effects upon illumination conditions typically used during photothermal therapy. In short, when the hollow nanoshells are immersed in citrate prior to functionalization with a Raman reporter, two dramatic changes were found upon irradiation: (i) a distinct blue-shift in the localized surface plasmon resonance, and (ii) an increase in the SERS intensity of the adsorbed molecule. This presentation explores our efforts to better understand the causes of these phenomena. This includes examining alternative reaction conditions, substituting citrate with other reducing agents and surface ligands, and importantly, exploring different templates to see if other types of metallic and hollow metallic nanoshells can also exhibit the same optical effects. By improving the SERS performance while simultaneously altering the optical properties of the nanomaterials during irradiation, this type of plasmonic nanoparticle may offer new methodologies for theranostics and photothermal therapies.

(SPR-01.5) Plasmonic Magnesium Nanoparticles in Action

Emilie Ringe¹, Vladimir Lomonosov¹, Thomas Wayman¹, Claire West¹, Elizabeth Hopper¹, Christina Boukouvala¹, Andrey Ten¹; ¹*University of Cambridge*

Localized surface plasmon resonances (LSPRs) have a broad technology potential as an attractive platform for surface-enhanced spectroscopies, light-enhanced chemical reactions, non-bleaching labels, hyperthermal cancer therapy, waveguides, and so on. One of the newest metals for plasmonics is magnesium. It is earth-abundant, biocompatible, and has a higher plasmonic quality factor than aluminum across the visible (and than gold and copper in the blue). In the past ten years, several fabricated magnesium structures have emerged, demonstrating the optical behaviors expected of plasmonic metals. Our group has chosen a different approach: we have developed colloidal, scalable batch and flow syntheses capable of size control from ~50 to 1000 nm. This enables us to study the fascinating size and shape-dependent optical, chemical, crystallographic and catalytic properties of these novel structures.

This talk will focus on the effect of the enhanced electric near-field created by LSPRs on Mg nanostructures. We will start by exploring, experimentally with EELS and numerically the distribution and intensity of near-field enhancement around various nanoparticle shapes, including their oxide layer. We will explore in depth the formation of the native oxide layer and the temperature and environmental stability of magnesium nanoparticles. Approaches to decorating the nanoparticles with other metals will then be discussed, followed by field enhancement results and studies of their photocatalytic properties in simple gas-phase hydrogenation reactions. Finally, proof-of-concept experiments probing the ability of this near-field to enhance chemical sensing will be reviewed.

22PLEN01: RSC Analytical Division Mid-Career Award

(PLEN-01.1) Sensitive and Selective Bioanalysis using SERS and SESORS

Karen Faulds¹, Duncan Graham¹, Matthew E. Berry¹, Anastasia Kapara, Samantha M. McCabe¹, Hayleigh Kearns; ¹*The University of Strathclyde*

Surface enhanced Raman scattering (SERS) is an analytical technique with several advantages over competitive techniques in terms of improved sensitivity and multiplexing. We have made great progress in the development of SERS as a quantitative analytical method. Many bioanalytical detection methods exist, with fluorescence spectroscopy tending to dominate, however SERS has the advantage that it is both sensitive and has the ability to multiplex which is limited when using techniques such as fluorescence. We have developed approaches to both identify and quantify the presence of multiple analytes within a mixture e.g. pathogenic DNA sequences, bacteria using SERS combined with data analysis techniques.

Here we demonstrate the development of new bioanalytical assays based upon SERS which have been used successfully for the detection of bacterial pathogens using modified SERS active probes. Biomolecule functionalised nanoparticles have been designed to give a specific SERS response resulting in discernible differences in the SERS which can be correlated to the presence of specific pathogens. In this presentation the simultaneous detection and quantitation of 3 pathogens within a multiplex sample will be demonstrated. We also explore the use of functionalized nanoparticles for the phenotypic screening of breast cancer cells and to study the effect of drug treatment on receptor status. The uptake of targeted versus non-targeted nanoparticles in breast cancer spheroids using a microfluidics approach will also be discussed. We have also recently published the use of nanoparticles functionalised with resonant Raman reporter molecule for the visualization of a 3D breast cancer tumour models at depth using Spatially Offset Raman combined with SERRS (SESORRS).

22PLEN01: SAS Ellis R. Lippincott Award

(PLEN-01.2) Advances in Interfacial and Voltage-gated Two-dimensional Infrared Spectroscopy

Martin Zanni¹; ¹*University Wisconsin-Madison*

This presentation will cover recent advances and applications of ultrafast two-dimensional infrared (2D IR) spectroscopy to interfacial chemical and biological systems. Interfaces are often difficult to study spectroscopically. There are relatively few molecules at the interface and interfaces are often buried under bulk solutions, making the interfacial signals small and difficult to observed from the bulk. Over the past 10 years, we have developed a series of technological advances that enable ultrafast 2D IR spectroscopy to be performed at interfaces. An overview of some of these advances will be given, with the focus of the talk on recent voltage-dependent measurements at electrode surfaces. The voltage-dependent studies are made possible by a new SEIRA substrate that supports both mid-IR surface plasmons, a voltage, and low background. Data and simulations will be shown that establishes that the structural motions of water at the interface are faster under an applied voltage. These results are important for understanding solvation reorganization energy at

interfaces and set the stage for many future experiments of voltage-dependent chemical and biological phenomena.

22AES01: Extraterrestrial Electrokinetics

Chair: Christopher Harrison

(AES-01.1) Single Molecule Methods to Seek Life As We Know It or Don't Know It Christopher E. Carr¹; ¹*Georgia Institute of Technology*

Humans now have the potential to seek direct evidence of life beyond Earth by targeting current or formerly habitable zones in our solar system. Life on Mars, if it exists or once existed, may be similar to life on Earth, or even related to us. At Ocean Worlds, energetic limitations imply ultra-sensitive approaches are needed, and we should target life as we know it or don't know it. Here I describe progress towards applying single molecule methods to meet these challenges, focusing on biological nanopores, solid state nanopores, and quantum-electron tunneling-based nanogap detectors for detecting and characterizing single molecules. While biological nanopores offer exquisite engineering of specific ionic current signals, facilitating sequencing of nucleic acids, non-standard base detection, and nascent peptide sequencing, solid state nanopores expand the range of detectable analytes, moving beyond assumptions of shared ancestry of common building blocks to facilitate a more agnostic approach to life detection. However, current solid state nanopore systems struggle to achieve single base resolution. Nevertheless, such nanopores still facilitate detection of different types of polymers and other sub-cellular structures, such as proteins or ribosomes. Nanogaps offer a complementary approach: in one variety, mechanically controlled break junctions can be used to create atomically sharp junctions at which tunneling currents can be used to characterize individual molecules (e.g., amino acids) or chemical moieties (such as individual bases of nucleic acids). Interaction events occur on millisecond timescales, orders of magnitude slower than solid state nanopore translocation times, a significant advantage for space applications. We have found that for amino acids, nanogap signals are larger for biogenic amino acids as compared to meteoritic amino acids. With improved electrokinetic control of molecular motion, nanogaps may facilitate sequencing of nucleic acid and other polymers. More broadly, biological nanopore, solid state nanopore, and nanogap technologies can be integrated to cover the full range of analyte size ranges from single molecules to whole cells. Future integration of such systems can address diverse Earth applications as well as facilitate seeking life beyond Earth, whether it may be life as we know it or as we don't know it.

(AES-01.2) Development and Optimization of Capillary Electrophoresis Instrumentation for Detection of Chemical Biosignatures on Future Life Detection Missions to Ocean Worlds

Mauro Ferreira Santos¹, Konstantin Zamuruyev¹, Aaron Noell¹, Maria Mora¹, Peter Willis¹;
¹*NASA Jet Propulsion Laboratory*

The search for chemical biosignatures on ocean worlds (e.g., Enceladus and Europa) will require automated instruments capable of performing remote measurements during in situ missions of exploration. The analysis of samples collected in such places will require

separation steps to “sort out” the target analytes prior to detection. In this context, capillary electrophoresis (CE) is a powerful and versatile separation technique that enables the analysis of a wide range of soluble organic compounds associated to life as we know it, as well as inorganic compounds that can serve as indicators of habitability. Here we report ongoing efforts to optimize the operation of a suite of portable CE hardware under development and test in our laboratory.

We have recently developed an automated CE system [1] based on custom-designed rotor-stator valves that allowed overcoming CE specific challenges: precise sample injection (using a rotary valve with fixed injection volume), HV isolation, and automation of all operational steps. This first prototype is compatible with multiple detectors and we demonstrate its performance by coupling this CE instrument to (i) mass spectrometry (MS), (ii) laser-induced-fluorescence (LIF), and (iii) contactless conductivity detection (C4D). Despite the simplicity of this design, we identified some limitations associated to sample injection that prevented achieving the maximum performance, especially for the sensitive LIF detection. We overcome these limitations by implementing a hydrodynamic injection approach (based on pressure and time) instead of using the rotary injection valve (fixed-volume). We were able to keep the simplicity and versatility of the design by maintaining the rotor-stator valves to perform all fluidic and pneumatic operations. This new approach increased significantly the “quality” of the injection and the overall performance of the CE instrument. Taking advantage of the flexibility associated to rotor-stator valves, we enhanced the capability of this CE system by enabling up to three-fold capillary multiplexing. This unique feature opens the possibility of having a single instrument with three different detectors (one in each capillary) or using all three capillaries in a single configuration for redundancy (to reduce single point failure).

References:

[1] Zamuruyev, K. et al. (2021) *Analytical Chemistry*, 93, 9647–9655.

(AES-01.3) Novel Deep Eutectic Solvents for the Fluorescent Labelling and Separation of Evidence of Past Life via CE-LIF

Jessica Torres¹, Christopher R. Harrison¹, Karen S. Campos¹; ¹*San Diego State University*

Novel deep eutectic solvents are used as separation and reaction media for future space missions.

The detection of present or past life on other worlds is a primary goal that spans across all of NASA’s enterprises. Planetary rocks such as those found on Mars are a primary target in our search for evidence of past life as these rocks may harbor traces of past microorganisms that have survived an oligotrophic environment. To increase the possibilities of detecting life, we aim to detect at the molecular level through amino acids. Amino acids are the fundamental building blocks of life and can be found in all three biological kingdoms on Earth, making these small molecules ideal targets towards future space missions.

Capillary electrophoresis coupled to laser induced fluorescence (CE-LIF) is a powerful analytical technique that has been widely used for the detection of biological samples, including amino acids. This technique can achieve sub part-per-billion limits of detection with low sample volume and consumption. While CE-LIF is an effective tool for future space missions, one limitation to its use is the sampling media. Solvents that are organic and highly volatile are not stable for long-term space exploration. Deep eutectic solvents (DES) are of particular interest as they are thermally stable and have low volatility. Our work currently involves utilizing DES as a solvent for non-aqueous fluorescent labelling for amino acid quantification and separation for the application on planetary rocks for future space missions.

This work presents the method development process for the non-aqueous fluorescent labelling of biologically important amino acids as signs of life. Amino acids derivatized using a DES based reaction were then separated using a DES chiral CE-LIF method up to low micromolar detection.

(AES-01.4) **Panel & Open Discussion**

22ATOM02: Single Cell & NP ICP-MS Part I

Chair: Alexander Gundlach-Graham

(ATOM-02.1) **Single Particle ICP-TOFMS: From Quantification to Interpretation**

Alexander Gundlach-Graham¹, Sarah E. Szakas¹, Stasia Harycki¹, Hark B. Karkee², Raven Buckman¹; ¹*Iowa State University*, ²*Iowa State university*

Single-particle inductively coupled plasma time-of-flight mass spectrometry (spICP-TOFMS) is used to analyze mixtures of nano- and micro-particles from a wide range of environmental sample types. With spICP-TOFMS, researchers aim to classify anthropogenic particle fractions based on multi-element signatures and to record the particle-mass (i.e. size) distributions and number concentrations of diverse particle types. However, the accuracy of these measurements requires an understanding of fundamental structure of spICP-TOFMS data and the development of consistent approaches to detect, classify, and quantify particles in natural samples. In this regard, improvement to the accuracy and robustness of calibration methods for spICP-MS and development of statistics-based methods to interpret recorded single-particle signals are critical.

In this presentation, I will discuss how the use of automated online microdroplet calibration improves both measurement throughput and accuracy of quantification of element masses in particles and particle-number concentrations. Following accurate spICP-TOFMS data acquisition and calibration, interpretation of the multi-elemental single-particle data sets is key. Our group is focused on understanding and predicting the structure of multi-elemental signal profiles from analyte nanoparticles as a function of particle composition, particle size, and measurement conditions (sensitivity, background, etc.). We have developed Monte Carlo models to test the impact of particle and measurement parameters and also

assess the match of these models to real data. We find that single-particle element profiles can be modelled very well as a convolution of particle size distribution and Poisson-distributed detection statistics. Using this model, we developed particle-specific detection limits based on Poisson statistics that allow us to set robust detection criteria for the classification of certain nanoparticle types as true single-element and/or multi-element nanoparticles. These particle-specific detection limits are predictable, can be applied to all spICP-TOFMS studies, and improve the use of multi-element fingerprinting to differentiate between classes of anthropogenic and natural nanoparticles. I will discuss the application of this approach for the classification and quantification of cerium- and titanium-rich anthropogenic and natural particles via spICP-TOFMS.

(ATOM-02.2) **Single particle/cell ICP-ToF-MS as a powerful tool in environmental and material research**

Björn Meermann¹; ¹*Federal Institute for Materials Research and Testing (BAM)*

Materials are key for our modern communities. In particular metals play important roles in all areas of our daily life - from building materials to high tech products.

Due to the increasing consumption of metals and corresponding waste production, an elevated release into the environment takes place. Furthermore, metals in direct contact with the environment undergo corrosion leading to a release into the (aquatic) environment. Thus, lifespan of products/buildings are substantially reduced – hence unnecessary economic costs arise. Thus, research in this regard is needed within the force field of metal/material - environment.

Evaluating environmental impact of materials as well as developing “safe” materials, new analytical methods are highly needed. One promising powerful tool is single cell-ICP-ToF-MS for multi-elemental analysis on a single cell/organism level.

Within this presentation the concept, strength as well as challenge of single cell-ICP-MS are briefly introduced. Two application examples are presented: (i) assessing the environmental impact of metals and (ii) the impact of the environment on metal-based materials and the derivation of potential environmental-friendly material protection strategies. These applications highlight the strength of new analytical approaches to explore the durability and safety of newly developed materials. Thus, analytical chemistry is one corner stone to transformation of modern society into circular economy (CEco).

(i) Diatoms are located at the bottom of the food chain. Thus, toxicological relevant metals taken up by diatoms possibly accumulate within the food web causing harmful effects. Diatoms are common test system in ecotoxicology. To investigate potential metal uptake and effects, we developed an on-line single cell-ICP-ToF-MS approach for multi-elemental diatom analysis. Our approach is a new potential tool in ecotoxicological testing for metal-based materials.

(ii) Next to classical corrosion processes, microorganisms are responsible for so called microbially influenced corrosion (MIC). MIC is a highly unpredictable process relying on

interaction pathways between cells and the metal surface. Shedding light on MIC processes and derivate potential protection strategies, we applied single cell-ICP-ToF-MS for MIC research on a single bacteria/archaea level. It turned out that microorganism are taking up particular metals from alloys - thus, single bacteria-ICP-ToF-MS will enable development of corrosion protection strategies.

(ATOM-02.3) Towards Normalization of Quantitative Single Cell ICP-MS Experiments

Maria Montes-Bayon¹, Roberto Alvarez-Fernandez Garcia, Juliana Severo Fagundes, Jörg bettmer¹, Zoltan Mester, Kelly LeBlanc; ¹*University of Oviedo*

Single cell analysis using elemental detectors, namely ICP-MS, can be conceptually derived from the single particle analysis ICP-MS experiments. Basically, once the cells are individually introduced into the ICP, the plume of ions generated can be directly measured using fast scanning mass analyzers in a sequential (quadrupole instruments) or quasi-simultaneous (time of flight instruments) way. However, cells are heterogeneous and fragile entities in comparison to nanoparticles. These characteristics increase the complexity of sample handling required to obtain reliable results from these experiments. Nowadays, a lot of work is done in the area of single cell ICP-MS using different quantification strategies. In order to obtain a normalized procedure for single cell inductively coupled plasma measurements (SC-ICP-MS), the quantitative characterization of the intra- and extracellular Se fractions of a Se-enriched yeast certified reference material SELM-1 has been carried out. The use of a standardized material (CRM) in this study will enable others to replicate, benchmark, and improve their procedures by using the same material.

In order to obtain a normalized procedure that can be applied to a wide analytical community, different sample preparation procedures have been studied. Best results were observed with a sequential washing procedure to isolate the cells from the extracellular Se content through which two fractions were obtained and analyzed by ICP-MS. After proving the cell integrity throughout the washing process by confocal microscopy, the Se intracellular fraction was determined by single cell-ICP-MS (SC-ICP-MS) as well as by acid digestion using microwave digestion. The extracellular Se concentration, has been directly determined in the washing solutions. The obtained results will demonstrate that with the appropriate sample preparation, SC-ICP-MS is a unique tool, which is capable providing quantitative information about intracellular and extracellular Se content in yeast at the individual cell level providing further granularity to speciation, toxicological and metallomics studies.

(ATOM-02.4) Size Determination of Nanoparticles by ICP-ToF-MS using Isotope Dilution in Microdroplets

Marcus von der Au¹, Sebastian Faßbender¹, Michail Ioannis Chronakis¹, Björn Meermann¹; ¹*Federal Institute for Materials Research and Testing (BAM)*

In recent years, the release of nanoparticles into the environment has increased significantly, not least because of their rapidly growing market share. Due to the strongly differing

properties of nanoparticles compared to bulk materials, the detection and evaluation of nanoparticles in the environment is an important issue for environmental analytics.

An established method for the detection of type and size of nanoparticles is ICP-MS. Here, nanoparticles are introduced as a suspension into the plasma of the mass spectrometer and the particles are recorded as "events". There are two main challenges that need to be addressed during the development of the method. i) classically calibration is carried out via liquid standards, which requires the determination of the transport efficiency for the sample introduction system and ii) coping with matrix effects, which may lead to ion suppression and thus to an underestimation of nanoparticle signal events.

Thus, to tackle the aforementioned challenges an isotope dilution (ID) approach with a microdroplet generator (MDG) as introduction system coupled to an ICP-ToF-MS was developed in this work. For ID, ICP-ToF-MS has the advantage that isotopic patterns can be measured in individual particles. Furthermore, the multi-elemental capability of the ToF mass analyzer allows also for the analysis of a second isotopic system to correct potential mass bias.

As a proof-of-concept study, platinum nanoparticles were analyzed by means of our new approach; it was shown that it is possible to correctly determine the size of the particles. To achieve this the $^{194}\text{Pt}/^{195}\text{Pt}$ ratio was applied; for (simultaneous) mass bias correction the $^{182}\text{W}/^{183}\text{W}$ ratio was used. By means of our approach, transport efficiency determination as well as external calibration becomes redundant. This makes the developed method very fast and robust. Furthermore, our on-line ID MDG-ICP-ToF-MS approach is easily applicable to further metal-based nanoparticle systems. A further beneficial field of application is single cell and organism analysis.

(ATOM-02.5) Metallic Environmental Particulate Matter Monitoring Using a Gas-Exchange Device Coupled to ICP-MS Run in Single Particle Mode

Chady Stephan¹, Aaron Hineman¹, Ruth Merrifield¹; ¹*PerkinElmer Inc.*

This is unique hyphenation of GED-SP-ICP-MS for environmental particle monitoring and analysis.

Atmospheric aerosols, or particulate matter (PM) are one of the main sources of climate change. However, the mixing state of aerosols and the influence of particle composition on particle size isn't well known. In this talk we will review a method that has been developed to directly analyze these aerosols on a single particle basis using a gas-exchange device (GED) coupled with quadrupole inductively coupled plasma–mass spectrometry (ICP-MS). A comparison between urban particulate matter collected in a city will be compared to aerosols from pristine locations, identifying elemental markers for anthropogenic aerosols and particles of natural origin will be presented.

Although the intention of a GED-SP-ICP-MS system is to eventually monitor the PM number concentration and composition in the environment in real time by injecting air

directly from outside the facility, we will also discuss the attempts to minimize sample preparation and collection. A minimal sample preparation is important to ensure the size and the composition of the particles aren't altered before analysis.

22AWD01: RSC Analytical Division Mid-Career Award Symposium Honoring Karen Faulds

Chair: Karen Faulds

(AWD-01.1) Knowledgeable Analytical Raman Enhancing Nanoparticles

Royston Goodacre¹, Howbeer Muhamadali¹; ¹*The University of Liverpool*

This talk is to celebrate Karen's award symposium – congratulations Karen on your Royal Society of Chemistry Analytical Division mid-career Award!

I will discuss our recent finds in SERS and learning from KAREN – which here we can define as Knowledgeable Analytical Raman Enhancing Nanoparticles – I will demonstrate how SERS can be designed to perform quantitative analysis of multiple analytes simultaneously. I will start by showing how SERS can be used for the detection and quantification of illicit drugs and their metabolites and how the SERS spectra relate to the functional structure of these drugs. I will then illustrate how multiple banned food dyes can be quantified within a single assay without the need for chromatographic separation.

(AWD-01.2) Translating Sensors from Feasibility to Future Product

Kristy S. McKeating¹, Kristy S. McKeating¹; ¹*Fitbit*

Over the past decade there has been a significant increase in the adoption of wearable devices as consumers become more conscious about the benefits of having access to important information about their health and fitness. As one of the key players in the wearable tracker and smartwatch field, Fitbit has continuously added to the arsenal of health information that is provided to a user, taking measurements that were traditionally performed in a lab or clinic onto the wrist while keeping the user experience simple and easy to use.

In a generation that is now accustomed to having access to continuous health information it is likely that this trend in awareness will continue and the next logical step will be to search beyond what can currently be measured on the wrist and delve into the wealth of health information that is contained within biological fluids. The challenge here becomes translating this next level of biosensor technology from the bench into a commercial device that is intended to be handled by a universal population, all while maintaining the accuracy, precision, and other performance metrics that are required to meet regulatory requirements.

This presentation aims to broadly discuss the key challenges associated with moving bench based research into a commercial product, while considering what the next generation of commercial biosensors will bring to the health and wellness space.

(AWD-01.3) Turning vibrational data into music

Colin J. Campbell¹, Colin J. Campbell¹; ¹*University of Edinburgh*

Communicating scientific ideas can be complicated, especially (but not exclusively) when communicating with the public. Music is an underutilised tool in communicating scientific ideas, but it has variables in its delivery such as tempo, rhythm and pitch which can be adopted to convey scientific meaning. In this presentation I'll give an overview of the approaches that I have used in turning scientific data and scientific ideas into pieces of music. In some of these examples the music plays a role of helping visualise the data, in others it helps support a narrative that explains the data. In this way, music can help start conversations about science in an engaging manner and help communicate scientific ideas with people who would otherwise feel marginalised by the subject matter.

Furthermore, making music can help reduce anxiety and stress – if you get nothing else out of turning data into music than an improved mental state, that's ok.

(AWD-01.4) Raman Spectroscopy and Semi-Supervised Learning for Improved Treatment of Patients Receiving HDR-Brachytherapy

Kirsty Milligan¹, Xincheng Deng¹, Ramie Ali-Adeeb¹, Phil Shreeves¹, Juanita Crook², Julian Lum³, Alexandre Brolo⁴, Jeffrey Andrews¹, Andrew Jirasek¹; ¹*University of British Columbia*, ²*BC Cancer, Kelowna, Canada*, ³*BC Cancer - Victoria*, ⁴*University of Victoria*

High-dose-rate-brachytherapy (HDR-BT) is an increasingly attractive alternative to external beam radiation-therapy (EBRT) for patients with intermediate risk prostate cancer. Despite this, no bio-markers or methods exists to monitor treatment response, and the changes which take place at the biochemical level in hypo-fractionated HDR-BT remain poorly understood.

Our group has shown that Raman spectroscopy (RS), combined with principal component analysis (PCA), can be used to identify biochemical changes associated with radiation exposure, however this has several limitations. Here we demonstrate an alternative approach in which a library of reference spectra containing individual cellular bio-components are used as inputs to group and basis restricted non-negative matrix factorisation (GBR-NMF). Using GBR-NMF we have successfully reproduced previously known metabolite response profiles in post irradiated MCF7 breast cancer cells such as those demonstrated for glycogen by Matthews *et al.*¹ using PCA. We here show that with GBR-NMF we now gain the ability to map profiles of other biologically relevant chemicals.

We have used the RS-GBR-NMF approach to elucidate biochemical expression patterns across a preliminary group of patients, identify clusters of individuals with similar profiles and shown some correlation of these expression profiles to the following pre-treatment clinical prognostic indicators; Gleason score, CAPRA score and Ki67 expression. The

ability to identify HDR-BT induced responses within individuals opens up a number of new treatment pathways that could be exploited to both increase the radio sensitivity of the tumour as well as the possibility to explore new, combination therapies.

Additionally, we have developed a workflow which combines transrectal ultrasound (TRUS) -multiparametric MRI (mpMRI) fusion images and spatially resolved Raman maps of biopsy cores. This method allows for analysis of normal tissue vs. diseased tissue response to HDR-BT as well as correlation of biochemical response profiles with HDR-BT dosimetry.

[1] Matthews Q, Isabelle M, Harder SJ, Smazynski J, Beckham W, Brolo AG, et al. (2015) Radiation-Induced Glycogen Accumulation Detected by Single Cell Raman Spectroscopy Is Associated with Radioresistance that Can Be Reversed by Metformin. PLoS ONE 10(8): e0135356. doi:10.1371/ journal.pone.0135356

(AWD-01.5) **SERS, SRS and Shenanigans**
Duncan Graham¹; ¹*The University of Strathclyde*

This presentation will take a light hearted look at the contributions made to the imaging of biological cells by Raman spectroscopy (including SRS) and the information gained in relation to various diseases. There will be a mix of science and humour aimed at the awardee who has dished it out for many years and now it's time for some levelling up of the playing field all within a professional scientific context.

22CHEM02: Advances in Chemometrics

Chair: Peter Harrington

(CHEM-02.1) **Building Concordant Ontologies Using KNARM (KNnowledge Acquisition and Representation Methodology)**

Hande Küçük McGinty¹; ¹*Kansas State University*

Recently, the increase and convenience in computational power enabled a rapid demand and need for data science applications. Research and development projects are seeking ontologies and machine-operable standardized vocabulary for approaches regarding cheminformatics and bioinformatics research. A continuous effort exists for creating applications that use ontologies and knowledge graphs across fields. In my research and through my volunteering efforts at ontology development groups, I was able to generate methodologies for creating and evolving bio-medical, food, and agricultural ontologies as well as utilizing them for applications that use machine learning algorithms in the backend. In this talk, I will give an overview of current directions, challenges, and possible future directions on building and evolving ontologies, and how using ontologies may help and accelerate the integration of knowledge representation across different domains.

(CHEM-02.2) **Data Tensorization for Better Curve Resolution of Exponential Mixtures**

Cyril Ruckebusch¹, Adrian Gomez Sanchez, olivier devos, Anna de Juan², Cyril Ruckebusch¹; ¹*University of Lille*, ²*Universitat de Barcelona*

Fluorescence imaging encompasses a set of non-invasive, highly specific and extremely sensitive analytical techniques, such as time-lapse fluorescence bleach rate imaging or fluorescence lifetime imaging microscopy (FLIM). Classically, multiexponential fitting is used to extract characteristic lifetimes for the fluorescent species present in the samples from the decay curves measured. In the context of multivariate curve resolution and linear data unmixing, the mathematical properties of exponential functions can be exploited to generate multiway data arrays (tensors) from the original matrices of decay curves. This strategy allows the application of multilinear factor decomposition algorithms such as PARAFAC or MCR-ALS with trilinearity constraints, to guarantee the uniqueness of the solutions obtained.

One approach for data tensorization is slicing, which consists of taking equally-sized subsets of a two-way data matrix of multiexponential curves at different time lags, reordering them into a three-way array [1]. By performing a trilinear decomposition of this data array by either PARAFAC or MCR-ALS with the suitable trilinearity constraint, the profiles obtained along the time mode are constrained to behave like mono-exponential functions. In principle, both approaches provide the same results but, since constraints can be implemented in a more flexible way in MCR-ALS (e.g., per profile), MCR slicing allows overcoming some limitations that may appear with pure trilinear slicing decompositions [2].

Another tensorization approach [3] is data kernelization, based on the principle that when an exponential signal is convolved by a kernel function, another exponential signal is yielded with a characteristic time that remains unchanged and, only its preexponential factor varies. For multiexponential decays, this means that the relative contribution of each fluorophore to the total signal changes, but not their lifetimes. Thus, by applying a set of distinct kernels to a given signal, trilinear data sets can be generated and subsequent trilinear decompositions can be performed.

We will show results obtained applying different data tensorization approaches to analyze time-resolved fluorescence data sets of different complexities taking advantage of the trilinear condition. The versatility of MCR-ALS hybrid bilinear-trilinear models for FLIM data will be emphasized.

(CHEM-02.3) **Generative Adversarial Linear Analysis**

Garth J. Simpson¹; ¹*Purdue University*

A linear algebra formulation is proposed for generative adversarial linear analysis (GALA), specifically designed to address overfitting common in chemometric analyses. In brief, GALA consists of iterations between generation of spectral “attacks” designed to co-locate with genuine spectra in a reduced-dimensional space, followed by discriminatory “defenses” to identify directions in spectral-space that isolate and remove the generated decoy spectra. In contrast to more conventional feature extraction strategies common in chemometric analyses, GALA seeks to identify the regions in spectral space in which the genuine data *aren't*, then exclude those locations from subsequent analyses. While inspired by generative adversarial deep convolutional networks (GANs), GALA relies on a wholly

unique mathematical foundation based on straightforward linear algebra manipulations that are both intuitive and computationally tractable for error propagation / uncertainty quantification. In an extreme example, GALA was applied for linear discriminant analysis (LDA) at full spectral dimension, for which the number of spectral-space parameters greatly exceeded the number of measured spectra (i.e., $p > n$). In this limit, LDA at full dimension is computationally nonsingular and exhibits extreme overfitting. Nevertheless, implementation of GALA enabled converged evaluation of LDA at full spectral dimension for both simulated and experimentally measured supervised Raman spectral datasets. In simulations designed to test sensitivity to unknown impurities, GALA provided improvements in classification accuracy relative to regularized LDA, PCA, and both naïve and optimized LDA+PCA. Strengths and limitations of GALA are critically discussed.

(CHEM-02.4) Using Chemometrics to Track Down the Source of Variance Between Authentic Botanical Samples

Jim Harnly¹; ¹USDA ARS

Chemometrics and multivariate analysis were used to evaluate the similarities and differences of authentic/vouchered botanical materials. In a previous study, authentic black cohosh (*Actaea racemosa*) from 3 excellent sources were found to show significant differences. A PCA scores plot of flow injection mass spectra (FIMS) showed considerable lack of overlap for authentic samples from American Herbal Pharmacopoeia, North Carolina Arboretum Germplasm Repository, and Strategic Sourcing. Inclusion of all authentic samples in a single model (SIMCA) provided a sensitivity of 69% and a specificity of 92% when compared to other *Actaea* species and commercial supplements. Cross validation showed poor results for sensitivity. While all the samples were vouchered, each was obtained from a different growing site, experienced different environmental factors, and were subjected to different storage locations and times. An examination of the counts for 1351 variables showed that 118 had relative standard deviations of less than 10% while the rest were around 35%. PCA based on only 118 variables improved specificity (88%) and cross validation and suffered only a slight decrease in specificity (89%). Weighting the FIMS counts by F values computed from multivariate ANOVA for *A. racemosa* and other *Actaea* species produced similar improvements in sensitivity, specificity, and cross validation. These results indicate that some components are more conserved and less influenced by environmental factors. PCA based on conserved variables improved sensitivity and did not appear to dramatically degrade specificity. Data collected with a non-targeted protocol can benefit by targeting specific variables. This could be considered a form of pre-processing.

(CHEM-02.5) Raman Spectroscopy Of Fish Blood as a Screening Test For The Lake Pollution With Perfluoroalkyl Substances (PFAS)

Luis Perez-Almodovar¹, Igor K. Lednev¹; ¹University at Albany, State University of New York

Raman spectroscopy combined with chemometrics was used to screen lake pollution's effect on fish health.

There is a critical need for a fast and onsite method for evaluating per- and polyfluoroalkyl substances (PFAS) pollution. The detection of PFAS in fish blood allows for the evaluation of time-integrated pollution as well as fish health. However, current PFAS detection methods are complicated, involve several steps, and are costly. As such, Raman spectroscopy with Chemometrics is investigated as a novel alternative method for detecting different concentrations of PFAS. Here, blood plasma obtained from smallmouth and largemouth bass located in several lakes with various concentrations of PFAS were analyzed. Partial least squares discriminant analysis combined with receiver operating characteristic curve analysis was able to separate the two groups (Low vs. High PFAS concentration) with 100% accuracy at the donor level during external validation. In addition, the Genetic Algorithm (GA) analysis identified the spectroscopic components responsible for the differentiation of fish classes. While further work is ongoing, these results demonstrate a potential for Raman spectroscopy as a successful method for PFAS detection, which can be essential as a diagnostic tool to predict fish health and monitor environmental contamination.

22CTP/EARLY01: Entrepreneurship in the Scientific Community

Chair: Alexis Weber

(CTP-01.1) Supporting Tech Transfer: The Funding Agency Perspective

Gregory Dutton¹; ¹*National Institute of Justice*

Implicit in federal funding support for scientific research and development is the promise that this public investment will ultimately result in tangible products for the public benefit—whether economic activity or other societal benefit. While objective means of selecting and funding of basic and applied research on its scientific merit are well established, it remains a challenge for federal funders to successfully bridge the gap between early and mid-stage R&D to the commercially viable transition of this technology to the marketplace.

The National Institute of Justice (NIJ) — the research agency of the U.S. Department of Justice (DOJ) — is a leading federal funder of research and development in the forensic sciences. NIJ maintains an external grant funding program that spans a broad range from fundamental research with the potential for application to forensic science, to the development of prototype devices, to the evaluation of novel instruments and methods. Examples of successfully transitioned lines of research at NIJ will be presented. The particular challenges of supporting technology transition at a small mission-oriented applied science agency will be discussed, with lessons for grantees and technology end users.

(CTP-01.2) Funding a Start-up and Navigating the World of Non-Dilutive Funding.

Jeffery Harrison¹; ¹*Pyrochem Catalyst Company*

Financing a small business comes in many forms, but it largely falls into two main categories: dilutive and non-dilutive financing. Dilutive financing requires an exchange of

equity, or ownership, in the company, while non-dilutive financing allows founders to retain full ownership. While non-dilutive seems like the easy choice, there are other considerations.

This talk examines the different types of non-dilutive funding, where to find it and the impact of timing on a company's growth and evolution. Of course there are strings attached to all types of financing, so we will also examine the pro and cons of non-dilutive financing. We also show how to use non-dilutive funds to maximize and leverage equity (dilutive) financing if and when that time comes.

(CTP-01.3) Time-Resolved Spectroscopy in Academia to a Successful Small Business Innovation Research Grant in Industry

Amy Scott¹; ¹*Beta Analytic*

This presentation focuses on time-resolved spectroscopic techniques typically used in academic projects and translated to industry through early-stage entrepreneurship. The pathway for National Science Foundation (NSF) funding through the small business innovation research (SBIR) granting system will be discussed along with the unique challenges starting up an R&D laboratory at Beta Analytic. The meticulous process of carbon-14 dating over the past 60 years has become a commercial enterprise and has led to innovations in scientific metrology and a demand for high-precision instrumentation in mass spectrometry. At Beta Analytic in Miami, Florida, the mass processing of carbon samples to graphite for analysis by high resolution accelerated mass spectrometry on part-per-trillion levels, has revolutionized our understanding of climate change, our planet, and human-kind. With over 600,000 carbon-14 samples processed that provide historical and archeological information dating ~43,500 years prior, Beta Analytic has been at the forefront of geochronology in the Quaternary era for the past 40 years as an ISO 17025:2017 accredited testing laboratory. However, not all geochemical samples are composed of carbon and the scientific window dating 43,500 – 200,000 years past does not yield a detectable carbon-14 signal. Martian landscapes composed of silicon and oxygen, sand dunes, mortar, ceramic fragments, sediments and rocks with fine grains of quartz and feldspar, provide a rich climatic history and paleoenvironment that are simply not detectable through carbon-14, accelerated mass spectrometry. Mummified bones can be dated using carbon-14 while the surrounding brick/mortar and sediments can be dated using Optical Stimulated Luminescence (OSL) and together create a more compelling time-line of burial sites than what either analytical dating technique alone can provide. Here, seed funding from NSF has allowed us to revisit optical dosimetry/dating techniques using advanced, pulsed laser methods 40 years after Beta's first investigations on thermoluminescence (TL) in the early 1980's did not yield success. Customers project a 10-fold increase in geological sampling with our proposed technological development thereby making a significant global impact simply through mass sampling and rapid analysis.

(CTP-01.4) Entrepreneurship for the Academic: the Good, the Baffling, and the Insanity

Alexander Scheeline¹; ¹*SpectroClick Inc.*

Business and academia have different goals, different values, and different measures of success. Nearly everyone becomes familiar with academic values through their education. Politics taints everyone's view of business values. Is profit the right variable to optimize? What about growth? Novelty (cast as either invention or innovation)? Efficiency? Staff size? Customer satisfaction? Environmental impact? This talk takes the viewpoint that moving from academic values to business values by becoming entrepreneurial casts the academic into unfamiliar territory where academic values are resisted. The consequences of different values leads to uncomfortable choices that are unavoidable in trying to build a company based on technology developed in academia. After explaining these tensions, two examples are presented, followed by advice to the would-be entrepreneur.

(CTP-01.5) Customer Led Design and “Failing Fast” in Hardware Design
Jonathon Speed¹; ¹*Keit Spectrometers*

Ever since its inception in 2001 the Agile mindset and methodology has revolutionized the way software is designed and delivered. It puts the principals of “people over product” into action and has shown significant improvements in both product quality and speed to market. Agile has high uptake in software development, but its implementation in hardware development is more limited. Here we present how Keit Spectrometers has used many of the principals of Agile to build and develop a product that actually delivers on what customers want and need, whilst also making money for the company.

We will give a brief history of Keit and an overview of our transition from traditional “waterfall” led project management to the Agile approach we use today. We will highlight the pivotal conversations we have had with customers over the last 8 years, and what this meant to product design at the time. We will also give examples of how we have “failed fast” (and an example of “failing slowly”) and the benefits this gives to getting the right answer as quickly as possible.

Lastly we'll explain how applications development has followed a similar approach of “failing fast” and finding commercially sustainable routes to implement highly technical spectroscopic equipment without the need to hire multiple PhD level chemometricians for commercial scale up.

22LIBS09: Geological Applications

Chair: Cécile Fabre

(LIBS-09.1) From Mineral Sources and Stalactites to Soils and Street Safety: LIBS Applications that Improve the Quality of our Lives

Nancy J. McMillan¹; ¹*New Mexico State University USA*

Laser-Induced Breakdown spectroscopy (LIBS) with chemometric analysis and AI offers potentially disruptive capabilities. Each LIBS spectrum contains an enormous amount of information, including concentrations of all naturally-occurring elements, isotopic ratios, and structural information. Several projects highlight the unique characteristics of chemometric analysis of LIBS spectra.

The geographic origin of gemstones is significant because: 1) gemstone value is in part based on provenance, and 2) agreements intended to limit human rights violations depend on accurate provenance determination. Traditional techniques comparing compositional similarities of relatively small sample sets on three-component chemical diagrams yield unsatisfactory results. Using LIBS, Kochelek et al. (2015) studied 569 rubies and sapphires from 21 locations in 11 countries with successful prediction rates of 97.9% (sapphire) and 95.4% (ruby) for the mine of origin. This approach was successful because both the number of samples and variables was large.

Identification of biotic and abiotic calcite cave formations permits of mapping the spatial distribution of not only where bacteria are currently active but also of where they were active in the past, a method potentially useful in the search for extraterrestrial life. Calcite samples from Fort Stanton Cave, New Mexico, USA, were analyzed with both desktop and handheld LIBS units; chemometric models resulted in successful prediction rates of 92% for both instruments.

Rapid measurement of key nutrients in soils could enable customization of fertilizers to provide only the nutrients missing in specific plots Omer et al. (2020) calibrated LIBS and visible-near infrared spectra to determine the concentrations of key nutrients in soils, demonstrating that LIBS provides good calibration curves.

Finally, aggregate materials used in roadways affect pavement quality. An automatic LIBS unit analyzed rock aggregate samples; chemometric analysis then pulled engineering properties from the calibrated spectra, some directly related to chemical composition. Successful models were developed to predict the specific gravity, expansion during freeze-thaw, and frictional properties of aggregates, as well as the identification of deleterious materials in aggregates.

These applications illustrate the power of this technology to change the way we think about the quality and sources of materials that we consume in our everyday lives.

(LIBS-09.2) LIBS to Fight Against Climate Change: A New Approach for the In-Situ Assessment of Carbon Capture in Geological Matrices

Josette El Haddad¹, Paul Bouchard¹, Christian Padioleau, Kim Renaud, Francis Vanier, Elton Soares de Lima Filho¹, Aïssa Harhira, Mohamad Sabsabi¹; ¹*National Research Council Canada*

Geologic CO₂ sequestration (GCS) has been identified as the most viable option for effectively reducing greenhouse gases in the atmosphere, mitigating global warming and worldwide climate change. CO₂ can be mineralized and sequestered in solid forms. Hence, geological matrices such as soil, mining residues (tailings) and man-made (industrial) waste, as well as directly available by-products (cements), have the potential to carbonate many Gt of CO₂/year. However, there is a technological gap for real-time monitoring of CO₂ sequestration by GCS processes. This gap has been identified quite early, being a

consequence of the lack of consistent results and methodologies for estimating CO₂ sequestration and storage capacity. Such a situation represents a major barrier to the deployment of carbon capture and sequestration efforts. For example, the optimization of the process of carbon capture requires the understanding of the composition of the geological matrices. Therefore, on-site monitoring of the carbon capture and of the factors, such as the physical characteristics (textures) and the chemical composition (minerals, elemental composition), involved in the sequestration of carbon is one of the keys allowing to accelerate the development of carbon capture by natural products. In the last several years, the National Research Council Canada (NRC) has worked with the Canadian SME Logiag on the development of the LIBS technology for the real-time analysis of various elements in agriculture soils. Furthermore, the NRC adapted the LIBS technology to perform automated in-field mineral characterization on rock samples in the mining sector. These combined development efforts by the NRC have actually spurred the capacities of the LIBS technology to support the fight against the climate change. In this perspective, the NRC is working on adapting a unique LIBS solution for the carbon capture assessment in geological matrices on-site. This solution is able to quantify the carbon content in these matrices as well as to characterize the factors that are involved in the carbon capture process. The methods developed and the progress achieved in the quantification of carbon and the identification of the factors involved in the carbon capture in geological matrices (such as soil, tailings and rocks) will be presented.

(LIBS-09.3) Contribution of LIBS to Mineral Resources: from Multi-Elementary Analysis to Mineralogical Mapping

Cécile Fabre¹; ¹*Universite de Lorraine / GeoRessources*

Mineralogical and petrographic studies require analytical methods capable to underline the repartition of major to trace elements within geological samples. The EMPA (Electron Microprobe Analysis) and μ XRF (X-Ray Fluorescence) conventional methods used for such investigation, but on restrictive zones, are on the verge to be reached by μ LIBS (Laser Induced Breakdown Spectroscopy) technique allowing rapid elemental cartography on thin rock sections or even larger samples in ambient conditions. This spectroscopic method with extremely fast acquisition speed (up to 1kHz), low detection limits especially for light elements (at sub-ppm level) is perfectly adapted to perform multi-elemental imaging of major to trace elements, and the possibility to scan large surfaces (several cm²) with a microscopic resolution (down to 15 μ m). In addition, since it is an all optical methods, it is rather easy to couple other characterization modalities such as optical, Raman or luminescence imaging. Mineral discrimination and relative elemental contents are also available with the portable LIBS tool on a millimeter observation area, directly on the surface of a rock outcrop or any rock section.

In this presentation, we will focus on recent research on the development of LIBS images for mineral resources through different topics: elemental mapping (quantitative or not), mineralogical discrimination or identification, correlation between multi-elemental results and molecular observations (i.e. Raman or luminescence) obtained with the same LIBS set-up, increase of spectra acquisition (up to kHz) with an extension of the sample surface.

Indeed, if the correlation of two/three elementary maps can be completed by *looking* (with our eyes) at the maps, when the number of data is too important, the contribution of chemometrics techniques can be essential.

(LIBS-09.4) Quantitative Analysis of Fluorine in Geological Samples with Handheld Laser-Induced Breakdown Spectroscopy

Gabrielle Lambton¹; ¹*Sciaps*

Accurate, repeatable, and rapid quantitative results of fluorine obtained with a handheld LIBS analyzer.

Laser-Induced Breakdown Spectroscopy (LIBS) is a useful tool for the analysis of geological samples due to the broad range of detected elements. This study utilized a portable LIBS system for the quantification of fluorine in geological samples. Empirical calibration models were built using an advanced PC-based software, Profile Builder, to collect quantitative data. This research looks at LIBS spectra collected in both He and Ar environments. The samples used include Cu-Au ore containing 340-72,500 ppm of F. Fluorine can be difficult to detect in a LIBS spectrum due to its high excitation energy, which results in weak emission of the atomic F lines. Fluorine is common in geological samples containing Ca. During the later stages of LIBS plasma formation, F will recombine with Ca to form molecules with characteristic molecular emission bands. This study used CaF molecular bands to build an empirical calibration for F and the results were compared to a calibration built using atomic F emission lines. The accuracy and limit of detection of F improved using CaF molecular bands versus F atomic emission lines. Signal-to-background ratios improved when plasmas were formed in He compared to Ar, whereas spectral intensities increased for plasmas formed in Ar. Calibrations made from plasmas formed in He (versus Ar) provided marginally better quantitative results for F. This research presents data showing that accurate, repeatable, and rapid quantitative results of fluorine can be obtained with a handheld LIBS analyzer.

(LIBS-09.5) In-Situ Multispectral Investigation of the Biogeochemistry of the Geldingadalir Lava Field

Kirby Simon¹, Pablo Sobron¹, Renata Barros², Giorgia Stasi², Aurélien Daussin³;
¹*Impossible Sensing*, ²*Geological Survey of Belgium, Royal Belgian Institute of Natural Sciences*, ³*Faculty of Food Science and Nutrition, University of Iceland, Reykjavík, Iceland / MATIS, Department of Research and Innovation, Reykjavík, Iceland*

Scientific payload demonstration in volcanic environments for astrobiological research understanding microbial colonization of fresh basalt.

The volcanic eruption in at Geldingadalir (Iceland) in 2021 is an ideal analog site for studying the biogeochemistry of volcanism on other planetary bodies, both those with active

(e.g. Io) and extinct (e.g. Mars) volcanic systems. The recent eruption enables comparative studies between the “fresh” lava field at Geldingadalir and older, inactive lava fields present throughout Iceland. Studying these systems provides insight into (1) the conditions necessary for microorganisms or other biotic materials to colonize barren environments and (2) how life transforms its environment over time. These investigations, while interesting in their own right for characterizing the biogeochemical diversity of Iceland’s landscapes, have implications beyond Earth in the search for extant or extinct life in our solar system.

To simulate planetary exploration missions, we deployed a suite of four handheld, low-SWaP (size, weight, and power), ruggedized spectroscopic instruments to enable in-situ investigation of the lava fields. We deployed a gamma ray spectrometer and laser induced breakdown spectroscopy (LIBS) probe for macroscopic and microscopic (respectively) assessment of the elemental composition of the natural samples; we used an ultraviolet (UV) fluorescence imager to investigate organic signatures present on the natural surfaces; and finally, we used a near-infrared (NIR) reflectance spectrometer for determining mineralogy and identifying hydrated bonding structures. These complementary measurement techniques enable a wholistic study of a samples’ biogeochemistry and have a direct path for mission infusion in planetary science, as various embodiments of these spectroscopic techniques have been used to study planetary surfaces for decades.

We collected co-registered spectroscopic measurements with all four instruments on several samples throughout the Geldingadalir lava field and at a control (i.e. inactive) field nearby. Additionally, we surveyed >10 surface and subsurface features throughout the lava field with one or more of the instruments. At the conclusion of this field campaign, we had collected >1000 UV fluorescence images, 10s of NIR reflectance and LIBS spectra, and >10 gamma ray measurements. Along with this, samples from the fresh and inactive lava fields were taken back to the lab for further investigation of microbial diversity using laboratory instrumentation.

22PAT05: PAT Coblentz: Machine Learning

Chair: Jim Rydzak

Co-Chair: Mike George

(PAT-05.1) Improving NIR Moisture Analysis through a Novel Synchronized, Automatic Calibration Data Collector

Adam J. Hopkins¹, Elena Hagemann¹, Scott Segro¹, Frank Koch¹; ¹*Metrohm USA*

We demonstrate a method for automatic NIR calibration curve data collection, generation, validation, and maintenance.

The analysis of moisture in chemical production and solvent recovery is commonly performed by coulometric Karl Fischer titration (KFT) in an offline wet chemistry lab. The time required for measurements and manual sampling often cause a lag between receiving data and process implementation. This results in either unnecessary drying steps or

insufficient drying, increasing costs. A better solution is online NIR measurement; however, the required moisture values are often too low for reliable offline KFT analysis and do not have enough variance to build a good a model – which is needed for NIR analysis. To overcome these challenges, we have developed a methodology for automatically creating NIR calibration curves, validating them, and maintaining them. In this study, we demonstrate how the system works by generating a calibration curve with warning and action limits for water in propylene oxide and independently validating that curve with additional material batches. We then show how the system can be deployed in a variety of configurations and be automatically updated in near-real time using a machine learning approach. This simulates production changes and the different implementations available for real-time process control.

(PAT-05.2) Sensor Agnostic Threat Anomaly Detection (ThreAD) for Explosives

Eric R. Languirand¹, Justin Curtiss¹, Darren Emge¹; ¹*U.S. Army DEVCOM CBC*

We show a real-time threat anomaly detection (ThreAD) algorithm that's agnostic to technique and sensor.

The Warfighter and first responders need to gather a lot of information and quickly determine if there are potential surface-based chemical threats that they need to be aware of. These chemical threats may take on different forms such as pharmaceuticals, explosives, or chemical warfare agents. However, most of the incoming data that may present itself will likely be innocuous and not affect the decisions that the Warfighter or first responder would need to make. Additionally, depending on the type of data acquisition, some efforts can be computationally cumbersome making real-time detection of threats difficult on a relevant time scale. Therefore, employing a Threat Anomaly Detection (ThreAD) algorithm may be an appropriate method for achieving real-time analysis of large amounts of spectral data by means of understanding if it is an anomaly and requires additional attention.

Herein we show using our ThreAD algorithm, which employs semi-supervised machine learning, can identify anomalies in the form of explosives-based ground contamination. The ThreAD algorithm works indirectly on the spectral content by utilizing the higher statistics and entropy of the probability distribution of the spectra. In this work we further show that our ThreAD algorithm is sensor agnostic as we demonstrate it with hyperspectral data (HyperThreAD) and Raman data (RamanThreAD) and that it has the potential to be more broadly applied to any time-series spectral-based signal.

(PAT-05.3) High Throughput Raman Monitoring of Downstream Bioprocess Purifications

Mark S. Kemper¹, Shamus Driver², Shaun J. Fraser¹; ¹*Tornado Spectral Systems*, ²*Tornado Spectral Systems*

Demonstration of higher time density tracking of protein chromatographic elutions

Downstream processing refers to the recovery and the purification of biosynthetic pharmaceuticals from biological sources, such as a fermentation broth. Of particular interest is the production and purification of therapeutic monoclonal antibodies (mAb) from mammalian cell lines in bioreactors. A multitude of sensors (pressure, temp, pH, UV-Vis etc) are used to provide bulk chemical and process information and High Performance Liquid Chromatography (HPLC) used to provide detailed, but insufficiently infrequent, quantitative molecular information. Raman spectroscopy provides highly detailed molecular information that can be used to quantify multiple products and impurities simultaneously with measurement times taking seconds instead of minutes. High-Throughput Raman spectroscopy can deliver considerable improvement in the Signal-to-Noise Ratio (SNR) compared to conventional Raman spectrometers. With improved SNR and signal throughput, speed of measurements are also improved. Combined with Partial Least Squares modeling, in-line measurements of protein can be made in under a minute with average prediction errors of less than 0.10 mg/mL. Use of High-Throughput Raman spectroscopy as a monitoring tool results in faster and predictively specific quantification of protein elution profiles from chromatographic separation processes. One can obtain highly detailed chemical information faster, with better signal-to-noise ratios, lower limits of detection and therefore, large improvements in downstream protein monitoring efficiency, yield, and purity.

(PAT-05.4) **Tablet API Determination Via Chemometric Analysis**

William Worley¹; ¹*JMP Statistical Discovery, LLC*

Chemometric analysis is growing field of study this contribution will feature unique capabilities regarding chemometrics.

Building an analytic workflow for any manufacturing process can be daunting. The goal of this presentation is to demonstrate the ease of building an analytic workflow from getting the data ready for analysis to analyzing the final product. The workflow will show steps for data visualization, multivariate analyses including clustering, and predictive modeling and optimization of the process. A chemometric modeling approach to quantify active API in a finished tablet will also be included in the workflow

(PAT-05.5) **ATR-FTIR and Chemometric Techniques in Solid Biofuels Application: Targeting the Bio-coke Product**

Supitchaya Cherdkeattikul¹, Yusuke Morisawa¹, Tamio Ida¹; ¹*Kindai University*

FTIR Spectroscopy implementation, as a non-destructive analysis for solid biofuels' quality control

Inquisition of solid biofuels as a coal substitution in the iron and steel industry, such as blast and cupola furnaces, increases rapidly due to the lack of sustainable carbon-neutral

solutions. The substitution rate of biofuels depends on their parameters, including the reduction rate, heating value, strength, and combustion properties. This study focuses on a bio-coke (BIC) solid biofuel produced under a sub-critical water state. The product is utilized in the cupola furnace foundry. The purpose of this study is to adapt the spectroscopy to the quality control process of the BIC product. ATR-FTIR spectra were collected from the side surface of the 12 mm diameter BIC produced from the mixture of Japanese cedar and additional lignocellulosic substances, pure cellulose, hemicellulose, and alkali lignin, at various mixing ratios. The spectra were analyzed using the principal component analysis (PCA) procedure. The principal component results were then transformed into discrete data to evaluate together with the BIC's technical parameters: compressive strength, particle density, and heating value, using the multiple regression model. The results show a significant correlation between the BIC's compress strength and the alteration observed in the 1800-1500 and 800-400 cm^{-1} regions. These alterations are due to the cleavage of conjugated C–O in quinies compound around 1650 cm^{-1} , and C=C aromatic skeletal at 1506 cm^{-1} of lignin compound and the increase of cellulose molecule. The result of the multiple regression analysis shows a substantial regression model. Two independent parameters that represent the spectra in 1800-1500 and 800-400 cm^{-1} regions explained 64.8% of variance in which predicted the compressive strength of BIC (Adj. $R^2 = .648$, $F(2,45) = 44.127$,) $p < .001$). The extension based on this result has the potential for the industrial BIC and solid biofuels' quality control procedure.

22PMA08: Bioprocess Materials and Methods

Chair: John Bobiak

(PMA-08.1) Rapid FTIR Method For Monitoring and Assessment of the Critical Quality Attribute of AAV Capsid Genome Packaging Contents

Yelena Pyatski¹, Yelena Pyatski¹, Kimberly Quinn¹, Rina K. Dukor¹; ¹*BioTools*

Adeno-associated viral (AAV) capsids are rapidly emerging vector technology for several novel gene therapy modalities (including transgene delivery and CRISPR gene editing modalities). The packing efficiency of genomes into AAV capsids can vary and depend on many factors during the industrial upstream production. Unpackaged genomes, partial genomes, and genomes packaged into AAV aggregates cannot be assumed to have the full efficacy of intact genomes packaged in a single intact, unaggregated capsid. Hence one of the most pressing critical quality attributes (CQA) during AAV manufacturing is proper assessment of genome packing in the capsid.

Many techniques on the market including electron microscopy, UV spectroscopy, column chromatography, ELISA, PCR-based and AUC are used for monitoring of genome packing efficiency. However, these techniques are expensive, high maintenance and hard to interpret. And in most cases the knowledge on empty / full ratio needs to be fast, easy and require small amounts.

FTIR possess advantage of being specific, sensitive, extremely easy to use, very fast and requires small sample volumes. It is label-free, dye-free, and standard-free. In this work, we present our results on nine serotypes with different promoters and demonstrate the

strengths of the technology for quantification of gene insert in various AAV serotype capsids.

(PMA-08.2) Characterization of Charge Tunable Nanoemulsions Stabilized by Cationic/Anionic Surfactant Mixtures

Konnor Jones¹, LAWRENCE SCATENA¹; ¹*University of Oregon*

Cationic/anionic surfactant mixtures can form stable, low charge nanoemulsions

Oil-water mixtures are present in many products that range from detergents to drug delivery systems. The functionality and stability of these products is intimately related to the properties of the oil-water interface, which can be tuned with a judicious choice of charged surface-active agents (surfactants). A surfactant's interfacial behavior can be unintentionally altered by electrostatic interactions with charged impurities. Hence, a fundamental understanding of how these interactions mediate the surfactant's adsorption is required. A *catanionic* surfactant (a mixture of cationic and anionic) is used to model a surfactant solution contaminated with charged impurities. Adjusting the mixing ratio and concentration of the catanionic surfactant tunes the strength of the electrostatic interactions between surfactants and influences their adsorptive behavior, compared to pure cationic and anionic surfactants. Vibrational sum frequency (VSF) reflecting and scattering spectroscopies are employed to examine the molecular structure and conformation of a catanionic surfactant (dodecyltrimethylammonium bromide/sodium dodecyl sulfate, DTAB/SDS) at the planar and curved nanoemulsion oil-water interfaces, respectively, as a function of mixing ratio. Concurrently, Wilhelmy plate tensiometry and zeta potential measurements are used to report on the interfacial population density and charge. Unlike the planar interface, nanoemulsion droplets are kinetically stabilized by strong electrostatic interactions. To understand why DTAB/SDS mixtures form stable, low charge nanoemulsions, we first examined the catanionic surfactant adsorbed at the thermodynamically stable planar interface. Insights garnered from the planar interface are extended to the nanoemulsion surface. The results of these studies aid in deriving a fundamental understanding of how catanionic surfactants influence the interfacial properties of nanoemulsions, which can lead to greater employment of catanionic surfactant stabilized systems.

(PMA-08.3) In-Line Lipidomics of Oil-Producing Yeast Cells for a More Sustainable Palm Oil Life Cycle

Karin Wieland¹, Mahmoud Masri², Jeremy von Poschinger², Thomas Brück², Christoph Haisch²; ¹*Competence Center CHASE GmbH*, ²*Technical University of Munich*

In-line fatty acid profile monitoring in yeast cells by Raman spectroscopy

Climate change with all its environmental implications, such as rising sea levels, the loss of biodiversity, or forest fires feeds the downward spiral of our planet Earth with devastating consequences. This alarming development gains additional momentum by deforestation of

tropical rainforests e. g. in favor of monoculture plantations, such as oil palm trees. In addition to the destruction of the forest's ecosystem, massive greenhouse gas emissions are associated with deforestation which negatively affect the palm oil life cycle. Nonetheless, the global palm oil demand is growing continuously as it has many favorable properties such as incredibly high yields (> 4x higher than alternative vegetable oils) and versatile applications in food, cosmetic, or fuel industry due to numerous beneficial chemical and physical properties.

To meet the market demand in a more sustainable way, the oily yeast fermentation industry holds great promise as alternative production process since the fatty acid composition shows remarkable chemical similarity to palm oil. One crucial parameter in palm oil production is the lipid composition inside oil-producing yeast cells as it determines product properties and quality. Typically, off-line GC analysis is employed for detailed fatty acid assessment. Here, we leverage in-line Raman spectroscopy to continuously monitor the lipid composition by investigating the changes in the ratio of unsaturated vs. saturated fatty acids. *Cutaneotrichosporon oleaginosus* – a widely used yeast with a high lipid content that can exceed 80 % - was employed for the fermentation. In-line Raman spectroscopy was applied in a non-contact way (ViewPort[®] sensor interface) for continuous, quantitative assessment of feedstock consumption, biomass growth, and lipid accumulation. Additionally, Gaussian curve fitting was used to mirror the dynamic profile of lipid composition over time ensuring high product quality. Monitoring this parameter almost in real-time allows for optimal process control with optimized product yields and ultimately represents an important step towards a more sustainable crude palm oil life cycle.

(PMA-08.4) Determination of Protein and Peptide Conformation and Orientation at Buried Interfaces in Situ in Real Time

Wen Guo¹, Tiewei Lu¹, Ralph Crisci¹, Satoshi Nagao², Tao Wei³, Zhan Chen¹; ¹University of Michigan, ²University of Hyogo, ³Howard University

New and systematic method to determine protein conformation and orientation at buried interfaces in situ

Proteins and peptides play important roles in a broad range of interface-related research and applications, such as biomedical materials, marine antifouling coatings, membrane protein functions, antimicrobial peptides, biosensors and biochips, etc. Therefore it is important to elucidate molecular structures of proteins/peptides at interfaces in situ in real time. It is difficult to do so due to the lack of appropriate tools. Recently, sum frequency generation (SFG) vibrational spectroscopy has been applied to study interfacial protein/peptide structures in situ. However, such studies have limitations and mainly determined the interfacial protein orientation based on the protein crystal structure. There are two major challenges: (1) How to determine complex interfacial protein structures with limited SFG measurements? (2) How to determine interfacial structure of a protein/peptide without crystal structure or with substantial changes of the structure at interface from the crystal structure? Recently, we developed a systematic approach to determine molecular structures

of proteins/peptides at buried solid/liquid and liquid/liquid interfaces in situ in real time. This methodology utilizes SFG experiments, molecular dynamics (MD) simulations, Hamiltonian SFG spectra calculations, protein structure predictions from the amino acid sequence, and isotope labeling. The Hamiltonian method is used to calculate SFG amide I spectra as a function of the protein orientation based on input protein conformations; the calculated spectra are then compared to the experimentally collected SFG amide I spectra to find the most likely protein orientation and conformation. We also demonstrated that with isotope labeling, we could greatly increase the independently measured SFG parameters to accurately determine the most likely protein structures at interfaces using protein Gb1 on a polymer surface as an example. The approach combining protein structure prediction from the amino acid sequence and MD simulation was used to study barnacle cement peptide adsorption onto polymers to understand molecular mechanisms of marine biofouling. The newly developed methodology is generally applicable, which can be used to study many different interfacial proteins/peptides at interfaces in situ, adding a powerful and unique tool to study interfacial biomolecules to the analytical toolbox.

(PMA-08.5) Microchip Electrophoresis for Single Cells Measurements of Oxidative Stress

Tyler Allcroft¹, Michelle L. Kovarik¹; ¹*Trinity College*

Microfluidic analysis reveals how single-celled organisms respond heterogeneously to stress, supporting a biological hypothesis.

Chemical cytometry is a form of single-cell analysis in which individual cells are lysed and their contents analyzed by microelectrophoresis. This technology complements more established forms of single-cell analysis, such as single cell sequencing and microscopy, by allowing quantitative measurements of diverse chemical species. We have applied this technology to study heterogeneity in the oxidative stress response of *D. discoideum*, a social amoeba. Cells were exposed to varying levels of oxidative stress using Rose Bengal dye and blue light to generate singlet oxygen. Oxidative stress in individual cells was determined using dihydrodichlorofluorescein as a reactive oxygen species indicator and carboxyfluorescein as an internal standard. The electrophoretic separation allows both dyes to be quantified in individual cells. Peak area ratios increased with blue light exposure, as would be expected for increased oxidative stress. Single-cell measurements demonstrated that variation within the population also increased with ROS exposure, supporting the biological hypothesis that a population of unicellular organisms samples a range of stress responses.

22RAM03: SERS 2

Chair: Sian Sloan-Dennison
Co-Chair: Royston Goodacre
Co-Chair: Zac Schultz

(RAM-03.1) SERS Based Monitoring of Bacterial Stress Responses

Peter J. Vikesland¹, Wei Wang¹, ASIFUR Rahman¹, Qishen Huang¹; ¹*Virginia Tech*

An improved understanding of bacterial inactivation mechanisms will provide useful insights for infectious disease control and prevention. We evaluated bacterial response to several inactivation methods using surface-enhanced Raman spectroscopy (SERS). The results indicate that changes in the SERS signal are highly related to cellular disruption and cellular changes arising after cell inactivation cannot be ignored. The membrane integrity of heat and the combination of UV₂₅₄ and free chlorine (UV₂₅₄/chlorine) treated *Pseudomonas syringae* (*P. syringae*) cells were severely disrupted, leading to significantly increased peak intensities. Conversely, ethanol treated bacteria exhibited intact cell morphologies and the SERS spectra remained virtually unchanged. On the basis of time dependent SERS signals, we extracted dominant SERS patterns. Peaks related to nucleic acids accounted for the main changes observed during heat, UV₂₅₄, and UV₂₅₄/chlorine treatment, likely due to their outward diffusion from the cell cytoplasm. For free chlorine treated *P. syringae*, carbohydrates and proteins on the cell membrane were denatured or lost, resulting in a decrease in related peak intensities. The nucleobases were likely oxidized when treated with UV₂₅₄ and chlorine, thus leading to shifts in the related peaks. The generality of the method was verified using two additional bacterial strains: *Escherichia coli* and *Bacillus subtilis* as well as in different water matrices. The results suggest that SERS spectral analysis is a promising means to examine bacterial stress response at the molecular level and has applicability in diverse environmental implementations.

(RAM-03.2) SERS of Cells: from Status to Physiological Process

Janina Kneipp¹, Janina Kneipp¹, Cecilia Spedalieri, Yiqing Feng, Vesna Zivanovic, Gergo Peter Szekeres; ¹*Humboldt-Universität zu Berlin*

Label-free SERS probing in cells has transitioned from proof-of-principle to the characterization of molecule-nanostructure and molecule-molecule interactions and cellular processes that involve a wide variety of biomolecules and cellular compartments. To gain a better understanding of cellular physiology and to harness the selectivity of SERS, different aspects must be addressed that involve (i) the use of good models, (ii) the development of appropriate targeting approaches, and (iii) the application of appropriate data analysis tools. We will discuss these aspects for the example of the endolysosomal environment of cultured animal cells, and show data that evidence the possibility to probe lipids, drug action, and the processing of proteins in the living cells.

(RAM-03.3) SERS-based Vertical Flow Assay on Plasmonic Paper for Point of Care Diagnostics

Jeremy D. Driskell¹, Eunice Ebbah¹, Richard Frimpong², Wongji Jang¹, Jun-Hyun Kim¹; ¹*Illinois State University*, ²*University of Alabama*

Plasmonic filter paper as a capture substrate in a SERS point of care test

Improvements in disease prevention and management may be achieved through better diagnostics and the ability to rapidly detect biomarkers of disease at the point of care. Immunoassays play a critical role in the analysis of biomarkers, yet these platforms are typically time- and labor-intensive with limited capacity for simultaneously analyzing

multiple biomarkers. Here we present an effort to address these challenges with the development of a vertical flow immunoassay optimized for surface-enhanced Raman scattering (SERS) detection. The method is based on a sandwiched system in which sample passes vertically through an antibody-functionalized plasmonic filter paper to selectively capture antigen. In a second step, a SERS-active nanoparticle passes through the plasmonic paper to selectively label the antigen. This vertical flow configuration affords reproducible flow of sample and label through the capture substrate to overcome diffusion limited kinetics and significantly reduced assay time, while SERS affords low level detection with multiplexing potential. In this presentation we will discuss the fabrication of the plasmonic filter paper, the role of plasmonic coupling between the sandwiched nanoparticles to optimize SERS detection, and the advantage of the vertical flow filtration for rapid and efficient sampling.

(RAM-03.4) Gold Nanoparticle (AuNP) Based Surface-Enhanced Raman Spectroscopy (SERS) Substrates for Sensitive Detection of Environmental Contaminants

SEJU KANG¹, Peter J. Vikesland¹; ¹*Virginia Tech*

Gold nanoparticle-based substrate can generate significant SERS signal enhancement and be applied for environmental analysis

Surface-enhanced Raman spectroscopy (SERS) has shown its great potential as an analytical technique with applications recently expanded to the environmental sector. It has been found that the localized surface plasmon resonance produced by plasmonic nanomaterials can provide an enhanced electromagnetic field where the Raman signal of an analyte is significantly enhanced, these locations are known as “SERS hot-spots”. Gold nanoparticles (AuNPs) have been widely used for environmental applications of SERS owing to their significant SERS enhancements and the chemical stability of gold. It has been shown that dense SERS hot-spots can be obtained by AuNP aggregation. In this study, we fabricated two different AuNP-based SERS substrates and applied them for the detection of environmental contaminants.

First, we optimized the slippery liquid-infused porous SERS (SLIPSERS) platform for the detection of antibiotic resistances genes (ARGs). The idea of SLIPSERS is to coat a porous Teflon membrane with a perfluorinated liquid that enables spatial concentration of a droplet containing AuNPs and target analytes upon free-spinning evaporation. The SLIPSERS approach generates significant numbers of SERS hot-spots and enabled detection at the 100 nM level of *mecA* and *intI1* gene segments - two genes of interest in the context of antibiotic resistance. Tree-based multiclass support vector machine classifiers were built to discriminate SERS spectra of 12 different gene sequences obtained by SLIPSERS: *mecA*, *intI1*, as well as analogues of *mecA* and *intI1*, respectively, with 2–10 base mismatches, and two random sequences.

Second, we fabricated porous Au supraparticles (i.e., several 100 μm sized agglomerates of primary nano-sized particles). The facile fabrication of the binary supraparticles was achieved by evaporating a droplet of AuNPs and polystyrene nanoparticles (PSNPs) on a superamphiphobic nanofilament substrate. Porous Au supraparticles were obtained through removal of the PS phase from the binary supraparticles by calcination. Six environmental contaminants (malachite green isothiocyanate, rhodamine B, benzenethiol, atrazine, adenine, and gene segment) were successfully sorbed to the porous Au supraparticles and their distinct SERS spectra were obtained. The limit of detection for the six contaminants ranged from ~ 10 nM to 10 μM . Overall, two AuNP-based SERS substrates were proven to be practical for environmental analyses.

(RAM-03.5) Biomimetic Transparent Nanoplasmonic Meshes by Reverse-Nanoimprinting for Bio-interfaced Spatiotemporal Multimodal Surface-enhanced Raman Spectroscopy

Aditya Garg¹, Elieser Mejia¹, Wonil Nam², Peter J. Vikesland¹, Wei Zhou¹; ¹*Virginia Tech*, ²*Virginia Tech*

Biomimetic nanoplasmonic meshes for spatiotemporal SERS analysis of living biosystems in targeted and non-targeted modalities.

Multicellular systems, such as microbial biofilms and cancerous tumors, feature complex biological activities coordinated by cellular interactions mediated via different signaling and regulatory pathways, which are intrinsically heterogeneous, dynamic, and adaptive. However, due to their invasiveness or their inability to interface with native cellular networks, standard bioanalysis methods do not allow in situ spatiotemporal biochemical monitoring of multicellular systems to capture holistic spatiotemporal pictures of systems-level biology. Here, we report a high-throughput reverse nanoimprint lithography approach to create biomimetic transparent nanoplasmonic microporous mesh (BTNMM) devices with ultrathin flexible microporous structures for spatiotemporal multimodal surface-enhanced Raman spectroscopy (SERS) measurements at the bio-interface. We demonstrate that the BTNMMs, carrying dense and uniformly-distributed nanolaminated plasmonic nanoantenna (NLPNA) arrays that support multiple hybrid localized surface plasmon (LSP) modes with spatial mode overlap, can serve as highly sensitive SERS devices (SERS enhancement factor $> 10^7$) for spatiotemporal multimodal SERS measurements in both targeted and non-targeted modalities. As a proof of concept demonstration, we show that the BTNMMs can simultaneously enable spatiotemporal SERS measurements for targeted pH sensing and non-targeted molecular detection to resolve the diffusion dynamics for pH, adenine, and Rhodamine 6G molecules in agarose gel. Moreover, the BTNMMs are employed as multifunctional bio-interfaced mesh SERS sensors to conduct in-situ spatiotemporal pH mapping and molecular profiling of *E. coli* biofilms. We envision that the biomechanical compatibility, transport permeability, and ultra-sensitive multimodal SERS capability of the BTMNNs can open exciting avenues for bio-interfaced multifunctional sensing applications

both in vitro and in vivo.

22RAM15: Raman Spectroscopy in Regenerative Medicine and 3Rs Research

Chair: Ioan Notingher

(RAM-15.1) Raman Microspectroscopy and Raman Imaging in Regenerative and Personalized Medicine

Julia Marzi¹, Katja Schenke-Layland¹; ¹*University of Tübingen*

As the field of regenerative and personalized medicine matures and cell- or materials-based therapies, as well as tissue-engineered products, play an increasing role in health care strategies, the need for novel enabling technologies for the artifact-free, real-time characterization of cells, (bio)materials, and tissue-engineered constructs in a more insightful, quantitative and preferably non-invasive manner becomes imperative. Raman microspectroscopy and Raman imaging have been established over the past 10 years to be suitable tools for the monitoring of the cell and tissue states in biological samples, assessing their biochemical and biomolecular structure marker independently. With the addition of machine learning and artificial intelligence technologies, Raman spectroscopy-based technologies will have the power to significantly improve diagnostic accuracy and speed, as well as provide reliable data for the improvement of cellular products and ATMP design optimization.

(RAM-15.2) Transcutaneous Raman Spectroscopy of Bones in Human Cadaver Hands

Andrew J. Berger¹, Christine Massie¹, Hani Awad¹, Emma Knapp¹; ¹*University of Rochester*

Multiple researchers have shown that near-infrared Raman spectroscopy can detect chemical signatures of bone in the human body transcutaneously. This offers the possibility of a Raman-based instrument providing non-ionizing bone assessments in primary care settings. In the context of bone health, such an assessment could help identify patients for dual-X-ray absorptionmetry scans, the gold standard for diagnosis of osteoporosis. By definition, however, a transcutaneous Raman spectrum also contains contributions from the overlying soft tissue. Using multi-distance spatially-offset Raman spectroscopy (SORS) and library-based spectral unmixing analysis, previous work in our group has produced estimates of murine tibial bones with significant fidelity to ex vivo measurements. To begin the process of adapting this approach for human subjects, we have performed a pilot study on twelve human cadavers obtained from the Anatomy Gifts Registry (Hanover, MD), including both osteoporotic and non-osteoporotic specimens as determined by X-rays of the wrist. We measured over phalangeal and metacarpal bones in the hand because of the thinness of the overlying soft tissue, the ease of access, and literature evidence that hand radiograph data correlate significantly with future fracture risk [Wilczek, Eur. Radiol., 2013, 23(5)]. A new optical system was developed to obtain simultaneous Raman measurements at source-detector offsets of 0, 3, and 6 mm, chosen based upon phosphate signal-to-noise ratios obtained in two previous cadaver hands over separations up to 8 mm. A trifurcated fiber-optic probe was constructed with different numbers of fibers to balance the different

signal strengths at the three offsets. Optical design software optimized the location and pointing direction for each fiber probe tip, and 3D printing enabled the probes to be held in place with a small form factor. For each of 12 hands, measurements were performed over three bones (proximal phalanx, intermediate phalanx, and metacarpal), leading to 108 bones in all. Spatially offset data were obtained for five source locations near the middle of each bone, in steps of 1 mm. We will discuss our spectral modeling using libraries of soft tissue and human bone, and unmixed bone spectra will be compared with ex vivo reference measurements.

(RAM-15.3) Raman Spectroscopy for Monitoring Native and Engineered Cartilage Health

Mads S. Bergholt¹, Mads S. Bergholt¹, Martin Hedegaard, Michael Albro, Elzbieta Stepula, Magnus Jensen, Brian Snyder, Anders R. Walther; ¹*King's College London*

Cartilage represents a complex connective tissue consisting mainly of aligned collagen, glycosaminoglycans (GAG)s and water. The tissue exhibits unique mechanical properties and changes in cartilage composition is associated with highly disabling diseases such as osteoarthritis. Here we present our recent development in Raman spectroscopy in regenerative medicine for monitoring the health of cartilage. We present different techniques spanning from ex vivo microscopic tissue characterisation, in vivo needle based Raman spectroscopy of joints to Raman tomographic reconstruction of tissue engineered cartilage constructs. Finally, we discuss possible applications in the pharmaceutical industry and for clinical diagnostics.

(RAM-15.4) Development of Bessel-beam illumination Raman microscopy for thick samples

Kazuki Bando¹, Shumpei Yabuuchi¹, Menglu Li¹, Toshiki Kubo¹, Ryosuke Oketani², Nicholas I. Smith¹, Satoshi Fujita¹, Katsumasa Fujita¹; ¹*Osaka University*, ²*Kyushu University*

We will discuss the observation of live spheroids with a Bessel Raman microscope.

As a non-invasive analytical method, Raman microscopy has been utilized in biological cell observation. In recent years, while there is a demand for the analysis of a single layer of cells cultured on a substrate, there is equally a demand for the analysis of samples closer to the living organism, such as spheroids/organoids and tissues, which have a three-dimensional structure. However, with a conventional Raman microscope, it is difficult to observe such thick samples due to the large background signal from the out of focus and the collapse of beam pattern at a certain depth. In order to observe Raman scattering of thick samples, we have developed a microscope that uses a Bessel beam as excitation light. Due to the characteristics of the Bessel beam, even deep sections can be observed with less aberration and keep the beam pattern. The Bessel beam was irradiated from the side to the detection surface, and the Raman scattered light from the Bessel beam was collected through a slit and spectrophotometer and detected by a CCD camera. We introduced an epi-line Raman illumination optical path with a common detection system. We observed the

same samples and compared and verified the detection capability in the direction of sample depth and in the magnitude of background light. It was found that the Bessel illumination optics improved the detection ability of the sample depth direction and the magnitude of the background light. As a result of observation of spheroids of living cells, the Bessel beam enables observation in a deeper direction with better contrast and suppression of background signals such as the substrate and culture medium. Raman imaging allowed us to discriminate the cells constituting the spheroid and the distribution of molecules inside the cells. This technology is expected to be used to evaluate the efficacy of drugs against spheroids/organoids and tissues in drug discovery, and to inspect the quality of regenerative medicine.

(RAM-15.5) Spectral CARS Signatures Identifies Intestinal Cell Types, Including LGR5+ Intestinal Stem Cells

Patrik K. Johansson¹, Katarina C. Klett¹, Chris Long¹, Sarah C. Heilshorn¹, Annika Enejder¹; ¹*Stanford University*

Label-Free Identification of Intestinal Cells Allows Studies of Stem Cell Differentiation and Proliferation.

The intestinal epithelium has a unique self-renewing capacity driven by Lgr5-expressing intestinal stem cells (ISCs) in the crypts, generating rapidly proliferating transit-amplifying cells that subsequently differentiate as they migrate up the villi. Unfortunately, no reliable antibodies currently exist for Lgr5 and, while powerful, GFP-labeled mouse models do not fully recapitulate human tissue. This makes investigations of human ISCs by traditional fluorescence microscopy challenging. Instead, label-free chemical imaging by nonlinear microscopy provides a way to identify cells based on the molecular compositions of their unique intracellular content. Characteristic lipid droplet accumulation and unsaturated fatty acid signatures allow for an alternative means of identifying highly proliferative cells and stemness, respectively.

In this work, we leverage spectral coherent anti-Stokes Raman scattering (CARS) microscopy to distinguish distinct cell populations in the intestinal crypt based on their intracellular accumulation of lipids and other biomolecules. Specifically, we explore whether unsaturated fatty acids are indicative of cell stemness and how stem cell differentiation might be accompanied by lipid droplet accumulation and increased lipid saturation. Using our custom imaging platform, we combine spectral CARS with traditional confocal fluorescence microscopy of cellular markers, allowing us to study the characteristic vibrational signatures of differentiated cells. Sections of the intestinal crypt from LGR5-GFP transgenic mice, allowed us to distinguish Paneth cells from LGR5⁺ ISCs, based on ratios of the CARS signals in the CH_x stretching and fingerprint regions. This is due to the dense and peptide-rich granules that fill up the Paneth cells, which have a strong signal at 2930 cm⁻¹ (CH₃ sym. stretch). In contrast, the LGR5⁺ ISCs have lipid droplets with a stronger signal at 2850 cm⁻¹ (CH₂ sym. stretch) and a relatively high degree of unsaturation, as determined by the ratio between 1650 cm⁻¹ (C=C stretch) and 1440 cm⁻¹ (C-

C stretch).

Based on these results, we conclude that identification of intestinal cells in the crypt is feasible by spectral CARS, which provides a path for label-free identification of LGR5⁺ stem cells. This would be a powerful approach for studying human ISCs that remain incredibly challenging to image, and therefore, investigate.

22SPECIAL02: Coherent Multidimensional Spectroscopy Symposium II

Chair: Wei Zhao

(SPEC-02.1) Local CO Behavior on Polycrystalline Pt Electrode Surface Using Compressive Sensing Sum Frequency Generation Microscopy (CS-SFGM) Combined with Electrochemistry

Steven Baldelli¹, Hao Li²; ¹*University of Houston*, ²*UH*

A new chemical imaging method based on compressive sensing and nonlinear optics

Compressive sensing sum frequency generation microscopy (CS-SFGM) was applied to study the CO adsorption on the polycrystalline Pt electrode surface. The crystal facets of heterogeneous polycrystalline Pt domains were determined by electron backscatter diffraction (EBSD). The SFG images stack obtained from broadband IR system contains the spectroscopic information of CO for in-situ adsorption behaviors study. Localized SFG spectra of C≡O stretching mode revealed the difference of CO behaviors on different crystal domains compared to the average spectrum. By controlling the potential applied on polycrystalline Pt surface, the Stark shift effect of each crystal domains was studied. The SFG spectra were fitted at every single pixel to map the peak wavenumber and Stark shift values distribution of C≡O stretching mode. The peak wavenumber map shows obvious boundaries between each domain while the Stark shift values map shows the non-local distribution across the whole surface. The preliminary results below suggest a possible mechanism for this effect and the outcome of the study will be an order-of-magnitude improvement in our understanding of the surface chemistry of practical, real systems, for both catalysis and corrosion. CO adsorbed to different crystal facets in a polycrystalline Pt electrode sample exhibits a vibrational resonance depending on the crystal plane to which it is bonded but is different than that of the externally prepared single crystal. However, the electrochemical vibrational Stark shift tuning rate dn/dV is very similar for each domain suggesting a leveling effect in the electronic structure and dependent on the localized grain structure arrangements. Thus while the study of single crystals is of great importance, the domains embedded in a polycrystalline matrix exhibit different surface chemistry, and this study represent a systematic controlled study to help bridge the ‘materials gap’ that exists in fundamental surface science studies.

(SPEC-02.3) Expanding Advanced Chemical Microscopy via Innovations and Commercialization

Ji-Xin Cheng¹; ¹*Boston University*

Advanced chemical microscopies including coherent anti-Stokes Raman scattering (CARS) microscopy, stimulated Raman scattering (SRS) microscopy, and mid-infrared photothermal (MIP) microscopy overcome the fundamental limits in conventional Raman and IR microscopy. Yet, it is a challenge to bring these advanced chemical imaging modalities to a broader community. In this presentation, I will discuss what we have done to accelerate the commercialization of CARS, SRS, and MIP microscopes.

(SPEC-02.4) Ultrafast Interconversion between Excitonic Valley States in Monolayer MoS₂ Due to Intrinsic Coupling

Greg Engel¹, Lawson Lloyd, Ryan Wood, Fauzia Mujid, Siddhartha Sohoni, Karen Ji, Po-Chieh Ting, Jacob Higgins, Jiwoong Park, Greg Engel¹; ¹*University of Chicago*

The valley pseudospin at the K/K' high symmetry points in monolayer transition-metal dichalcogenides (TMDs) has potential as an optically addressable degree of freedom in next-generation optoelectronics. However, intervalley scattering and relaxation of charge carriers leads to valley depolarization and limits practical applications. Using circularly polarized 2D electronic spectroscopy, we show that exchange coupling causes extremely fast (sub 10 fs) interconversion between valley states. The coupling element linking these valleys seems to be intrinsic and not a function of grain size suggesting a fundamental origin for the coupling. Indeed, the coupling element is strong enough that it challenges the notion that spin-locked valleys are good eigenstates for this material.

(SPEC-02.5) Stimulated Raman Excited Fluorescence: Combining the Best of Two Worlds

Wei Min¹; ¹*Columbia University*

The pursuit of a hybrid spectroscopy that combines the superb sensitivity of fluorescence and the high chemical specificity of Raman scattering has lasted for 40 years, with multiple experimental and theoretical attempts in the literature. It was only recently that the stimulated Raman excited fluorescence (SREF) process was successfully observed in a broad range of fluorophores. SREF allows single-molecule vibrational spectroscopy and imaging in the optical far field without relying on plasmonic enhancement. Herein we will first review the historical efforts that lead to the successful excitation and detection of SREF, followed by the underlying physical principles, then the remaining technical challenges will be discussed, and, at last, the future opportunities in this old but yet newly emerged spectroscopy are outlined.

22SPSJ03: Frontiers of Vacuum, Far, and Deep-Ultraviolet Spectroscopy I

Chair: Yusuke Morisawa

(SPSJ-03.1) Electrochemical Far- and Deep-Ultraviolet Spectroscopy Applied for Organic Semiconductor/Ionic Liquids Interfaces

Ichiro Tanabe¹; ¹*Rikkyo University*

Attenuated total reflectance spectroscopy applied for the far- and deep-ultraviolet regions (ATR-FUV-DUV spectroscopy) enables to obtain electronic excitation spectra in various materials. Recently, we have developed new electrochemical system into the ATR-FUV-DUV spectroscopy, and investigated the electronic states of ionic liquids and organic semiconductors under voltage application.

(SPSJ-03.2) **Direct Observation and Attribution of the Vertical Transitions of the**
Nami Ueno¹, Yusuke Morisawa², Yukihiro Ozaki³; ¹*Kobe University*, ²*Kindai University*,
³*Kwansei Gakuin University*

The electronic transitions of the aqueous solutions became easier to directly observe due to the development of the ATR-FUV as a novel experimental method. And the ATR method is particularly good at measuring the concentrated aqueous solutions. These concentrated solutions, especially so-called molten salts called the Water-in-salt (WIS) and the Hydrate melt (HM), have recently attracted a great deal of attention. This is because WIS and HM are expected to be the high performance (safe and large potential window) electrolytes in Li secondary batteries.

In this study, the electronic transitions of HMs consisting of LiTFSI and LiBETI reported by Yamada et al. were observed using ATR-FUV. These results show that the electronic states of the concentrated solution have significant differences from the pure water. Furthermore, the dependence on the concentration of aqueous salts and the combination of the Li salts were compared to confirm the feature of the electronic transitions of general Li-salt solutions and super concentrated Li-salt solutions. Now, we focused on the changes in the electronic transitions of water molecules in pure water and ultra-concentrated aqueous solutions, elucidated the origin of the electronic changes and assigned the transitions of ultra-concentrated aqueous solutions. In a brief conclusion, transitions of water molecules showed a large blue shift (close to 0.4eV), and the reason for this blue shift is caused the direct interaction of water with the Li⁺. And the transition energy of water molecules was distinguished by the water molecules belonging to the first or the second hydration shell in Li-salt aqueous solutions.

(SPSJ-03.3) **Changes in Electronic States of Saturated Cyclic Compound with Six-Membered Rings**

Yusuke Morisawa¹; ¹*Kindai University*

Electronic structure of σ molecular orbitals and their hyper-conjugation play an important role in the conformation preference and reactivity in saturated-organic molecules. A nonbonding electronic orbital introducing the saturated structure is interacted with the σ orbitals. We have already published the alternation of FUV spectra by the methyl substituted on the axial position of cyclohexane due to effect of hyper-conjugation.[1] The FUV spectra of cyclohexane and methyl cyclohexane in neat liquids showed a band with central wavelengths near 155 and 162 nm. The FUV spectra of dimethyl cyclohexane with two methyl substituents at the equatorial positions (trans-1,2-, cis-1,3-, and trans-1,4-) and trans-decalin had similar features to those of cyclohexane. The decrease in intensity and the

longer-wavelength shift of the 155-nm band for dimethyl cyclohexane (with one methyl group at the axial position) and cis-decalin were observed. The reason for such a large spectral alternation for the axial substitution may be the increase in the orbital energy of the occupied orbital which has its electron density concentrated at the axial C–H bond. Regarding the effect of the hyperconjugation of C–C and C–H σ orbitals, the second perturbation energies of the interaction between C α –H α x and C β –H α x were estimated for molecules by natural bond orbital (NBO) analysis.

The electronic states of tetrahydropyran and its methyl and hydroxy substitute in the gas phase were investigated a long time ago using far ultraviolet spectroscopy to reveal the interaction in the saturated cyclic carbohydrate. However, detailed assignment of the transition and relation between electronic transition and structure has not been investigated. Moreover effects of hydrogen bond on the σ orbitals through the hyper-conjugation have rarely been investigated. Here we show the FUV spectra of tetrahydropyran and its methyl and hydroxy derivatives in the liquid states by attenuated total reflectance spectroscopy. The difference in FUV spectra in the introduction of oxygen was compared with cyclohexane and its derivatives.

[1] Morisawa, Y.; Higaki, Y.; Ozaki, Y., *J. Phys. Chem. A* 2021, 125, 37, 8205–8214

(SPSJ-03.4) Label-Free Autofluorescence-Detected Mid-Infrared Photothermal Microscopy

Aleksandr Razumtcev¹, Minghe Li¹, Garth J. Simpson¹; ¹*Purdue University*

AF-PTIR microscopy enables label-free photothermal mid-IR microscopy with greatly improved sensitivity

Autofluorescence-detected mid-IR photothermal (AF-PTIR) microscopy enabled chemically-selective label-free imaging of an active pharmaceutical ingredient (API) within a commercial solid dosage form. The ubiquity of aromatic moieties in aromatically conjugated small molecule APIs supports their native autofluorescence in the UV region. Temperature-induced changes in the UV-autofluorescence quantum efficiency were shown to be a sensitive indicator of localized absorption of mid-IR radiation. The spectral masking approach designed for an array of 32 independent quantum cascade lasers (QCLs) enabled to perform fingerprint region AF-PTIR microspectroscopy with high spatial resolution dictated by two-photon excited UV-fluorescence (TPE-UVF) microscopy. The difference in vibrational spectra enabled the discrimination of API particles from a TPE-UVF active excipient complementing the results that could be obtained by TPE-UVF microscopy alone. Furthermore, AF-PTIR was shown to complement the results of conventional optically-detected photothermal microscopy (O-PTIR) by enhancing the selectivity and signal-to-noise characteristics. Label-free mapping of API distribution has the potential to assist in designing and production of powdered heterogeneous solid dosage forms of pharmaceutical materials.

(SPSJ-03.5) Imaging Molecular Diffusion And Adsorption Through Nanoporous Silica Particles: Exploring Molecular Transport In Chromatography Separations

Hong Bok Lee¹, Max Lei Lei Geng¹; ¹*University of Iowa*

The origin of the dispersive chromatographic band was explored by imaging diffusion and adsorption kinetics.

Fluorescence fluctuation spectroscopy and fluorescence correlation spectroscopy (FCS) can reveal the hidden heterogeneity in molecular populations through accumulation of single molecule signals at high spatial-temporal resolution. Although nanoporous particles have been utilized in a wide range of chemical and biomedical applications such as catalysis, chemical separations and biosensors, the heterogeneity of the molecular transport processes has not been adequately assessed. There are still many unraveled microscale molecular transport phenomena and parameters interfacial area between mobile and stationary phase in the chromatography packing particles. The heterogeneous distribution of adsorption and desorption sites of silanol group is accounted for its problematic low quality of resolution for the chemical separation. In this talk, I will present the imaging of diffusion and adsorption kinetics in the nanoporous silica particle with line-by-line scanning FCS and computer simulation. The method of our confocal scanning technique gives an advantage to probe the molecules which diffusion regime is close to the realistic intraparticle condition of industrial liquid chromatography. The hypothesis of the study is that the molecular dynamics inside chromatographic silica particle pores are affected by both Brownian motion and adsorption kinetics where those populations are heterogeneous through the silica particle; the diffusing molecules will appear to adsorb and desorb at hydrophobic layers or silanol groups on the pore surfaces. To test this hypothesis, the translational diffusion and desorption rates of fluorescent probe in the silica particle were measured by the line-by-line scanning FCS. We constructed both diffusion coefficient and desorption rate constant images, and the result of images showed us the heterogeneity of those kinetics over the silica particle which implies the origin of the dispersive chromatographic peak resolution. The mathematical model for fitting and interpreting the autocorrelated fluctuation data was tested by generating Monte Carlo simulation combining Brownian motion and random stopping-departing motion. Overall, the effects of heterogeneous chromatographic environments on diffusion and adsorption were explored, and these studies are essential towards developing a better understanding of the molecular transport in chemical separation science.

22ATOM06: Single Cell & NP ICP-MS Part II

Chair: Antonio Montoro Bustos

Co-Chair: C. Derrick Quarles Jr.

(ATOM-06.1) Determination of Proteins in Single Cells by Inductively Coupled Plasma-Mass Spectrometry using Metal Nanoclusters as Labels of Specific-Recognition Reactions

Beatriz Fernandez¹, Paula Menero-Valdés¹, Ana Lores Padin¹, C. Derrick Quarles Jr.², Montserrat García³, Héctor González-Iglesias⁴, Rosario Pereiro¹; ¹*University of Oviedo*, ²*Elemental Scientific, Inc.*, ³*Instituto Oftalmológico Fernández-Vega*, ⁴*IPLA-CSIC*

Heterogeneity of cell populations is well-known in all biological systems. In fact, cells of the same type, even under equal physiological conditions or external stimuli, may differ in the level of biomolecule expression. Furthermore, it can be difficult to assess and correctly interpret possible differences between cell populations, unless biological systems are investigated on a quantitative cell-by-cell basis. Therefore, there is a need for innovative analytical techniques that allow for the determination of elements and especially biomolecules in individual cells.

Single cell ICP-MS (sc-ICP-MS) has demonstrated huge potential for the determination of elemental compositions in individual cells. The quantification of endogenous cellular proteins still remains a challenge since it is necessary to perform an immunoassay in cell suspensions by using an immunoprobe conjugated with an elemental label. However, this step can compromise cell integrity and so far a limited number of studies have reported on the combination of an immunoprobe and sc-ICP-MS for protein analysis. Furthermore, the applications related to quantitative protein analysis are very scarce. There is another critical point for absolute protein quantification; the amplification factor (i.e., the number of elemental labels per immunoprobe) must be known, thus requiring the use of a well characterised immunoprobe for each protein of interest.

In this work, the use of metallic nanoclusters (NCs) as elemental labels is reported for the determination of proteins in individual cells by sc-ICP-MS. The proposed methodology is based on immunoassays performed in a cell suspension by using AuNCs and IrNCs conjugated to protein-specific antibodies for the sequential determination of cytosolic and membrane proteins in human retinal pigment epithelial cells. sc-ICP-MS allows a fast cell-to-cell determination of target proteins within cell cultures, although subcellular distribution cannot be obtained. In our experiments, the spatial distribution of the target proteins was evaluated in individual cells by laser ablation (LA) ICP-MS. Thus, sc-ICP-MS and LA-ICP-MS are here proposed as complementary techniques for the characterization of proteins in cell cultures: determination of target proteins will be performed by sc-ICP-MS with high-throughput cell analysis while the distribution of the proteins at the single cellular level will be performed by LA-ICP-MS.

(ATOM-06.2) Finding Small Particles in Complex Samples: Recent Advances of spICP-MS

Carsten Engelhard¹, Darya Mozhayeva¹, Annika Schardt¹, Johannes Schmitt¹, Ingo H. Streng¹; ¹*University of Siegen*

In this presentation, recent advances in plasma spectrochemistry for the detection of nanoparticles will be reviewed and some contributions from our laboratory to this field will

be presented.

In the first part, recent developments in inductively coupled plasma mass spectrometry (ICP-MS) instrumentation for nanomaterials characterization in complex mixtures will be reviewed. The current state-of-the-art in single-particle (sp) ICP-MS instrumentation for the detection and characterization of single nanoparticles (NP) as well as remaining challenges will be discussed. While millisecond dwell times were used in the advent of spICP-MS, the use of microsecond dwell times helped to improve nanoparticle data quality and particle size detection limits. Further to this development, we could show that a custom-built high-speed data acquisition unit with microsecond time resolution (μ sDAQ) can be used to successfully address issues of split-particle events and particle coincidence, to study the temporal profile of individual ion clouds, and to extend the linear dynamic range by compensating for dead time related count losses.

In the second part, our next generation DAQ for spICP-MS will be presented, which features nanosecond time resolution. The capabilities of a low-cost, home-built nsDAQ unit will be discussed, which include a fundamentally different and dedicated data processing workflow for highly sensitive nanoparticle detection, size determination, and concentration calibration.

(ATOM-06.3) **Analysis of nanoparticles in food by single particle ICP-MS**

Katrin Loeschner¹, Katrin Loeschner¹, Janja Vidmar², Luisa Hässmann¹; ¹*Technical University of Denmark*, ²*Jožef Stefan Institute*

Food is one major source of exposure of consumers to nanoparticles (NPs) via the oral route. NPs that could potentially be present in food are, e.g., naturally occurring NPs, engineered NPs (intentionally added or released from food contact materials), incidental NPs formed and released during preparation or production of food, and anthropogenic NPs entering the food chain via the environment. In addition, food additives, such as titanium dioxide (E171), iron oxides / hydroxides (E172) and silver (E174), can release or contain a fraction of particles at the nanoscale. Studies are required to determine the level of NPs in food to allow an assessment of consumer exposure. Further, it is necessary for food control purposes to measure whether intentionally added engineered NPs and food additives containing small particles can be distinguished from the background level of other NP types.

In this context, inductively coupled plasma-mass spectrometry in single particle mode (spICP-MS) is a promising technique for the screening of food samples for the presence of metal-containing NPs, as it provides information on particle size and concentration with high sensitivity. Further advantages of spICP-MS are fast analysis, relatively simple sample pre-treatment, and easy implementation in state-of-the-art ICP-MS instruments, which otherwise can be used for metal analysis and speciation. We demonstrated the potential of spICP-MS as a screening technique by investigating the presence of 8 types of NPs (containing silver, aluminum, chromium, copper, iron, silicon, titanium, or zinc) in 13 different food products (DOI: 10.1021/acs.jafc.0c07363). The highest mass concentrations of NPs were found in the samples with food additives which are known to contain a fraction of NPs. The source of NPs in food that could not be related to any labeled food additive (or

other ingredients known to contain NPs) was in many cases difficult to identify. Particle sizes and mass concentrations determined by spICP-MS were based on the assumption of a certain particle composition, density and shape. Confirmatory techniques for particle characterization, such as electron microscopy in combination with elemental analysis, should be applied if more accurate values are required.

(ATOM-06.4) **Single Cell ICP-MS (SC-ICP-MS) to Study the Uptake and Apoptotic Status of Nanoplatinum (IV) Treated Cells**

Lucía Gutiérrez-Romero¹, Elisa Blanco-González¹, Borja Gallego-Martínez², René Rodríguez-González², Maria Montes-Bayon¹; ¹*University of Oviedo*, ²*FINBA*

The use of nanocarriers to improve the selective transport of chemotherapeutic agents represents a large area of research. Among the multiple types of nanocarriers, iron oxide nanoparticles are of great interest due to their biocompatibility in the body, fewer side effects and their good incorporation into the cytosol by endocytosis. In addition, the synthesis of ultrasmall iron oxide nanoparticles coated with tartaric and adipic acids has permitted their conjugation with metalodrugs like cisplatin and its analogues. Cisplatin, cisdiamminedichloroplatinum (II), is a well-known drug that is widely used in the treatment of different types of cancer. However, its intravenous administration is hampered by important limitations such as high toxicity, nonspecific interactions with plasma proteins that generate drug inactivation, as well as limited drug uptake by tumor cells in resistant models. For this reason, the use of cisplatin (IV) prodrugs has been one of the proposed alternatives to overcome such limitations. Platinum (IV) complexes are more resistant to ligand substitution reactions than platinum (II) agents, minimizing unwanted side reactions with biomolecules prior to binding its cellular target (DNA).

Herein, we explore the capabilities of biocompatible ultrasmall iron oxide nanoparticles coated with tartaric and adipic acid, to be directly conjugated to the cisplatin (IV) prodrug. After the characterization of the nanodelivery system by different techniques (HPLC-ICP-MS, TEM...), we employed single-cell inductively coupled plasma (SC-ICP-MS) to study their incorporation in different cell models (A2780 and OVCAR-3). The quantitative results of the incorporation levels will be illustrated in this work. The evolution of the nanodelivery system within the cell cytosol will be also illustrated as well as the capabilities for DNA platination using complementary analytical strategies. In addition, by conjugating the capabilities of SC-ICP-MS to cell sorting (by flow cytometry), we will show that is possible to compare the level of platination in the different cell populations (viable, apoptotic and necrotic cells). Furthermore, the use of SC-ICP-MS for addressing the efficiency in the nanodelivery of Pt (IV) prodrugs in more complex 3D cellular models like spheroids and organoids will be also illustrated aiming to have a more complete picture on the behavior of the nanosystem.

(ATOM-06.5) **Online Microdroplet Calibration for the Quantification of Metal and Metal Oxide Nanoparticles in Organic Matrices**

Stasia Harycki¹, Alexander Gundlach-Graham¹; ¹*Iowa State University*

Method for accurate quantification of nanoparticle masses and number concentrations in organic matrices with spICP-TOFMS

Single particle inductively coupled plasma time-of-flight mass spectrometry (spICP-TOFMS) is an established method for nanoparticle characterization. This technique simultaneously measures the (multi-)elemental compositions of nanoparticles in liquid samples at rates up to thousands of particles per minute. However, ICP-MS is highly susceptible to matrix effects, which makes the quantification of nanoparticles in complex matrices a challenge.

When volatile organic solvents are nebulized via conventional pneumatic nebulizer, small droplets that are readily transported into the ICP are formed, which increases plasma-uptake relative to that of aqueous samples. At the same time, organic solvents can cause attenuation of absolute elemental sensitivities from the ICP-MS. These sample-introduction-related and plasma-related matrix effects can hinder the quantification of particle number concentration and element mass in individual nanoparticles. To overcome matrix effects, we use online microdroplet calibration. In this setup, nanoparticle-containing samples are introduced to the plasma along with monodisperse microdroplets containing known element mass amounts. Microdroplet signals are used to calibrate absolute element sensitivities and determine accurate particle sizes regardless of sample matrix. To overcome matrix-dependent plasma-uptake rates, a plasma-uptake standard element is spiked into both microdroplets and nanoparticle samples, which allows for accurate particle number characterizations.

I will present the use of an online microdroplet calibration system for the measurement of metal and metal oxide nanoparticles in several organic matrices including methanol, ethanol, and isopropanol. With microdroplet calibration, 100 nm gold nanoparticles were accurately sized within the manufacturer's value in up to 98% ethanol, even while particle signals were attenuated by 96%. Without the droplet calibration, the gold nanoparticles were undersized by 71%. Online microdroplet calibration also enabled the determination of the particle number concentration within the manufacturer's value even with more than a four-fold increase in sample transport caused by the ethanol matrix.

22AWD09: SAS Ellis R. Lippincott Award Symposium Honoring Martin Zanni

Chair: Martin Zanni

(AWD-09.1) **Dynamics of Protein Molecular Recognition via Vibrational Spectroscopy**
Megan Thielges¹, Megan Thielges¹; ¹*Indiana University*

As the molecular machines of a cell, proteins move to execute their functions. However, to fully uncover the role of protein dynamics, the population of and interconversion among

multiple states, experimental approaches for their characterization must contend with both the complex spatial heterogeneity of proteins and the rapid interconversion of potentially important states. To address these challenges, our group combines the inherent temporal resolution of linear and two-dimensional infrared spectroscopy with the spatial resolution afforded by site-selective incorporation of vibrational reporter groups with frequency-resolved absorptions. This enables capture of even rapid dynamics at local environments in proteins. I will share how we have applied this approach to investigate the functional role of dynamics in protein molecular recognition and catalysis.

(AWD-09.2) Commercialization of Ultrafast 2D Spectroscopy: How a Spectroscopy Startup Grew from the Basement into a Company

Chris T. Middleton¹; *¹PhaseTech Spectroscopy, Inc.*

I will describe my experiences co-founding an ultrafast spectroscopy company as a spin-off from a university research laboratory - the ups & downs and the lessons learned along the way. PhaseTech makes research-grade instruments for ultrafast spectroscopy including 2D spectrometers, femtosecond pulse shapers, and detection systems. We sell to universities and government research laboratories all over the world. Our company started as a spin-off from the Zanni group at University of Wisconsin-Madison, with nights/weekends in 40 square feet of space in the corner of my basement. We now occupy a > 4000 square foot facility with multiple full-time employees and customers all across the U.S., Europe and Asia. I will talk about how we've been able to do that as well as many of the mistakes I've made along the way.

(AWD-09.3) Structural Transitions of FUS Protein Within Liquid-Liquid Phase Separated Droplets Probed by Light Scattering and 2DIR Spectroscopy

Arnaldo Serrano¹, Anna Zepeda, Dean Edun; *¹University of Notre Dame*

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are severe and deadly diseases. In recent years, the discovery of several genetic causes to these conditions has revealed that a key mechanism of disease progression may lie in liquid-liquid phase separation (LLPS) of a number of peptides and proteins. Among these is fused in-sarcoma (FUS), a protein known to aggregate in ALS disease. Using pulse-shaper enabled techniques for simultaneously observing in-situ light scattering and two-dimensional infrared (2DIR) spectra, we observed changes in the amide-I 2DIR spectra of FUS associated with folding and subsequent aggregation within LLPS droplets. Using exciton scattering simulations of the 2DIR spectra, we report on the likely secondary structural transitions induced by LLPS and interpret these results in light of proposed disease models.

(AWD-09.4) Measuring Protein Structure and Dynamics on Nanoparticle Surfaces via 2D IR Spectroscopy

Lauren E. Buchanan¹; *¹Vanderbilt University*

We are developing new isotope-labeling strategies to determine and localize structural changes in nanoparticle-bound proteins with two-dimensional infrared (2D IR) spectroscopy. First, we address questions which remain regarding the inherent sensitivity of

isotope-labeled modes to local changes in α -helicity, such as terminal fraying; the origin of spectral shifts (hydrogen bonding vs. vibrational coupling); and the ability to definitively detect coupled isotopic signals in the presence of overlapping sidechains. Next, we discuss how transition dipole strength spectra combined with site-specific isotope labeling allows us to distinguish discrepancies in aggregate structure that arise from seemingly minor modifications, such as N-terminal acetylation. Finally, we explore how metal nanoparticles affect the aggregation of human islet amyloid polypeptide.

(AWD-09.5) **Applications of IR Spectroscopy from PIKE Technologies**

Kent Gundlach¹, Jenni Briggs¹, Andy Bean; ¹*PIKE Technologies*

Advancements in spectroscopy are often a result of a synergistic union between researchers' needs, and the engineers' imagination of what is possible. For over 30 years, PIKE Technologies has been developing innovative accessories and optical components to help users reach their goals. Here, we will review PIKE's capabilities and detail selected PIKE products, including those developed through customer collaboration.

22IR04: Mid-IR Lasers and Detectors as Enabling Technology for New Sensing Schemes

Chair: Markus Brandstetter

(IR-04.1) **Rapid Vibrational Circular Dichroism – Opportunities through the combination of External Cavity Quantum Cascade lasers and balanced detection**

Daniel-Ralph Hermann¹, Georg Ramer¹, Bernhard Lendl¹; ¹*TU Wien*

Maximizing the advantages of tunable Quantum Cascade lasers for low noise Vibrational Circular Dichroism

Chirality is an important aspect of the chemical and biological world, being present prominently in our own body. Consequently, it has implications for the pharmaceutical industry, as the absolute configuration of an analyte influences its therapeutical index. Due to this fact, Vibrational Circular Dichroism (VCD), which can assess the chirality of an analyte in solution, has become a routinely used tool when dealing with active pharmaceutical ingredients. However, while VCD, which operates in the IR spectral region, is broadly applicable, it suffers from a signal intensity about 10^5 times weaker than classical IR absorbance spectroscopy. This circumstance necessitates long measurements (several hours) to collect reliable VCD spectra.

Quantum Cascade Lasers (QCL) are tunable mid-IR lasers, offering intense and highly polarized emission in the IR region. For classical IR spectroscopy, QCLs already outperformed classical FT-IR in terms of limits of detection and signal-to-noise ratios (SNR) even in demanding aqueous solvent systems. QCL based VCD instruments are also able to leverage the inherently linearly polarized light as it eliminates the need for a polarizer. However, QCLs come with disadvantages not present in thermal light sources, mainly thermal drifts and pulse

to pulse intensity fluctuations. These phenomena introduce noise into the measurement system, possibly offsetting the advantages gained from employing a QCL.

To counter these drawbacks, we combined a QCL based VCD setup with a balanced detection system. In balanced detection, the laser beam is split into two beams, which are directed onto two detectors. The detector signals are then subtracted. Laser noise, present in both channels, is compensated, while the analyte information, only present in one channel, is still accessible. After adapting this scheme to VCD, we reported a noise level decreased by a factor of 4 compared to single detector systems. Compared to classical FT-IR, we report a decrease in noise by a factor of at least 2 within a third of the measurement time. With this increase in measurement speed, QCL based VCD has the potential to be used as monitoring tool for more dynamic process, like chiral reactions or protein folding.

(IR-04.2) **Mid-Infrared Hyperspectral Single-Pixel Imaging Microscopy**

Alexander Ebner¹, Paul Gattinger¹, Ivan Zorin¹, Christian Rankl¹, Markus Brandstetter¹;

¹*Research Center for Non-Destructive Testing GmbH*

Mid-infrared (mid-IR) microscopy provides spatially resolved chemical information through the spectroscopic evaluation of characteristic absorption features for each pixel. Since molecular information is accessible under ambient conditions in a contactless, label-free and non-destructive way, mid-IR microscopy has evolved as an essential tool in scientific and industrial laboratories. Although this technique brings valuable benefits in chemical analysis, conventional mid-IR microscopy based on Fourier-transform infrared (FTIR) spectroscopy struggles with the limited spectral brightness of thermal sources leading to a trade-off between signal-to-noise ratio, spatial resolution and acquisition time. The modern counterpart – tunable mid-IR laser-based microscopy – on the other hand is typically associated with extensive costs, which are also driven by the application of high-end mid-IR cameras. To overcome these limitations, we demonstrate an alternative approach for hyperspectral microscopy that, to the best of our knowledge, is the first to exploit a single-pixel (SPI) imaging approach for microscopy in the mid-IR spectral range. SPI applies a spatial light modulator, such as a digital micromirror device (DMD) with frame rates of up to 40 kHz, to mask an image with a time-varying pattern. The masked image is then collectively projected onto a single-pixel detector for synchronized intensity measurements, which allows for the reconstruction of the image. Especially for the mid-IR spectral range, this concept brings decisive advantages: image acquisition in the ms time regime, use of an inexpensive single-pixel detector with excellent detectivity and exploiting the multiplex advantage – known from FTIR spectroscopy – in the spatial domain. Additionally, in the presented SPI microscope, we utilize the dispersion by diffraction of mid-IR wavelengths at the DMD micromirrors (in the range of 10 μm) and apply a single mid-IR modified DMD as a hyperspectral imaging tool. Diffraction limited 64 \times 64 images are acquired and reconstructed in 450 ms and 162 ms per wavelength, respectively, thus, drastically improving the sample throughput in mid-IR chemical and biomedical imaging.

(IR-04.3) Introducing Temperature-controlled Desorption Separation by Nanoelectromechanical Photothermal Infrared Spectroscopy

Niklas Luhmann¹, Robert G. West², Raphael Pliessnig², Josiane P. Lafleur¹, Silvan Schmid²; ¹*Invisible-Light Labs*, ²*Institute of Sensor and Actuator Systems - TU Wien*

Over the past decade, nanomechanical infrared spectroscopy (NAM-IR) has demonstrated exceptional sensitivities for the detection of chemical compounds down to single molecules. In this approach, the analyte is sampled directly onto a nanomechanical resonator using standard aerosol techniques. Depending on the molecular specific absorption of the sampled compound, the radiation of an infrared light source is used to locally heat the resonator. By measuring the resulting resonance frequency change with respect to the scanning wavelength of the infrared laser, one can generate an infrared spectrum of the sampled compound.

Here, we introduce a novel technique for desorption-based separation of picogram sample based on NAM-IR spectroscopy. This method shows the capability of combining the analysis of infrared spectra and thermo-gravimetric properties simultaneously with the same device. This allows for the identification of IR active vibrational modes and the extraction of desorption enthalpy for single compounds and mixtures in parallel. To demonstrate the potential of this technique, two xanthins, caffeine and theobromine, were separated by thermally controlled desorption under vacuum conditions and the process was monitored by NAM-IR. By applying a global analysis tool designed for ultra-fast spectroscopy, on the spectro-temporal data of a mixed solution, it was possible to extract the spectrum and related desorption lifetime of both chemical compounds out of a single measurement with femtomole sensitivity. We expect that this technique will find a wide range of applications for the physicochemical characterization of complex samples in various fields, from environmental analysis to pharmaceuticals.

(IR-04.4) Quantum Cascade Laser Dual-Comb Spectroscopy in Solid, Liquid, and Gas Phase Measurements

Markus Mangold¹, Raphael Horvath¹, Jakob Hayden¹, Pitt Allmendinger¹; ¹*IRsweep AG*

QCL frequency combs have proven ready for rapid analysis in a broad range of applications

Dual-comb spectroscopy is a powerful direct absorption spectroscopy technique and has attracted considerable attention in recent years. Quantum cascade laser (QCL) frequency combs have made frequency combs in the mid-infrared available for a broad range of applications. In this presentation, we will analyze how QCL dual-comb spectroscopy can be adapted to the various challenges posed by different applications.

Absorption spectroscopy of gases is important in industrial quality control, environmental studies, as well as for basic research applications. In the gas phase, absorption features are often narrower than the inherent line spacing in QCL frequency combs of typically 0.3 cm^{-1} . We will present strategies how to “fill the gap” between the widely spaced frequency comb

modes resulting in spectra covering more than 50 cm^{-1} with a spectral resolution in the 10^{-4} cm^{-1} range.

Spectroscopy of fluids is widely used for chemical reaction monitoring. In fluid phase, a major challenge for mid-infrared studies is the strong background absorption, which often limits the optical path length in fluid samples to only a few micrometers. We will show how microsecond time-resolved infrared stopped-flow spectroscopy has increased sensitivity because of the long interaction path achievable with high power QCL frequency combs.

Finally, infrared spectroscopy of solids is relevant in applications such as stand-off detection of hazardous substances or heterogenous catalysis. Solid samples are often analyzed in diffuse reflection configuration. Such measurements are challenging because of the low intensity of the scattered light reaching the detector. We will show how QCL frequency combs can be used to analyze minute changes in solid samples with microsecond time resolution.

In summary, we will show that QCL dual-comb spectroscopy has become a viable tool for many types of analytical chemistry applications.

(IR-04.5) New Approaches to High-Sensitivity QCL-IR Spectroscopy of Proteins in Water

Young J. Lee¹, Seong-min Kim¹, Yow-Ren Chang¹; ¹*National Institute of Standards and Technology*

Novel optical techniques enable higher-sensitivity QCL-IR spectroscopy to characterize proteins in dilute aqueous solutions.

Infrared (IR) absorption spectroscopy is a powerful tool that can quantify complex biomolecules and their structural conformations. However, conventional approaches to protein in aqueous solutions have been significantly challenged because the strong IR absorption of water overwhelms the limited dynamic range of the detection system and thus allows only a short path length and a limited concentration sensitivity. Here, we demonstrate an adaptive solvent absorption compensation (SAC) approach can improve the concentration sensitivity and extend the available path length by distinguishing the analyte signal over the full dynamic range at each wavelength. We present QCL-based IR absorption spectra of hydrated proteins from $>100 \text{ mg/mL}$ to $<0.1 \text{ mg/mL}$ protein concentration, allowing a two to three orders of magnitude enhanced signal-to-noise ratio in the amide I band compared to the non-SAC results. We also present new advancements in spectral range and speed of the absorption measurement. This simple optical technique can be applied to other absorption spectroscopies of analytes in strongly absorbing solvents, allowing for enhanced sensitivity without changing the detection system.

22IR07: Photothermal Session II

Chair: Rohith Reddy

(IR-07.1) O-PTIR and Raman Spectroscopic Imaging for the High Resolution Elucidation of Breast Microcalcification Heterogeneities.

Nicholas Stone¹, Pascaline Bouzy¹, Keith Rogers², Robert Scott, Iain Lyburn, Eleanor Cornford, Charlene Greenwood, Jayakrupakar Nallala¹; ¹*University of Exeter*, ²*Cranfield University*

Breast microcalcifications have been demonstrated to provide key information on surrounding tissue physiology and pathology. Vibrational spectroscopy has been at the forefront of this discovery and elucidation of key pathological differences continue. Recently, the development of techniques such as O-PTIR (optical probe - photothermal infrared spectroscopy) has enabled an order of magnitude improvement in spatial resolution for IR spectroscopic mapping. This combined with Raman (possible with the Photothermal mIRage system) has enabled the further discovery that calcifications exhibit huge variations in spatial distributions of various organic and mineral components, further opening the way for more detailed exploration to enable a clearer understanding of microcalcification formation mechanisms and the relationship with invasive cancers.

Here we will describe the use of the mIRage with a 785 nm probe. This has been able to provide simultaneous NIR-Raman spectra for the first time. The combined co-located data enables clear validation of spectral assignments which can sometimes be tricky by single modalities alone.

(IR-07.2) Correlative Spectroscopic Analysis of Buccal Cells: O-PTIR (Far Field IR, Raman) and Superresolved Fluorescence Imaging

Kathleen M. Gough¹, Sabine Mai¹, Mustafa Kansiz², Gorkem Bakir¹, Atacenk Basic¹, Benoit Girouard¹, Curtis Mensforth¹, Darryl Dyck³, Rohith Reddy⁴, Chalapathi Gajjela⁴; ¹*University of Manitoba*, ²*Photothermal Spectroscopy Corp*, ³*Lumiere Microscopy*, ⁴*University of Houston*

Buccal cells are of neuroectodermal origin and may reflect pathological changes of brain cells. The nuclear architecture of buccal cells is altered in Alzheimer Disease (Mathur et al. 2014; Garcia et al. 2017), but molecular changes within the interchromatin regions and cellular alterations remain unclear. Our goal is to analyze correlated fluorescent, infrared and Raman spectra and images, at less than 300 nm spatial resolution, to improve our understanding of genomic instability and changes in nuclear architecture related to disease processes. The proposed correlative imaging method is not only feasible but will be clinically relevant for a simple, safe, objective measure for staging AD that will save healthcare costs and optimize patient treatment strategies. Infrared and Raman spectra provide complementary, molecular fingerprint information that can facilitate the identification of spectroscopically relevant clinical biomarkers. To this end, proof-of-principle experiments have been performed on Human Oral Mucosal Epithelial cells (H-6234, Cell Biologics Inc. USA), mounted on substrates suitable for spectroscopy and

imaging. Single IR wavelength images of intact cells and individual simultaneous O-PTIR and Raman spectra were collected with a mIRage™ IR Microscope operating under PTIR studio software (v. 4.3). For cells on glass coverslips, IR images and spectra (1800-900 cm) were collected in counter-propagating mode, with the IR QCL laser illuminating from below and the 532 nm probe laser from above, viewed through an oil immersion objective (UPLFLN100XO2, Olympus Corp), 1.3 NA, compensated for 0.17 mm glass, with matching refractive index of oil. Cells on CaF₂ windows were imaged with O-PTIR in co-propagating mode with transmission detection at single wavelengths between 3000-2700 and 1800-900 cm⁻¹; simultaneous IR and Raman spectra were obtained in the same spectral regions for IR, and continuously between 4475-900 cm⁻¹ for Raman. Following spectroscopy with mIRage™, samples underwent staining and correlative fluorescent imaging for nuclei and cytoplasmic organelles. Additional fluorescent imaging was performed on parallel cells from the same preparation. Image registration permitted correlative overlay images of multiple intact cells with all three spectroscopies, at spatial resolutions of less than 300 nm.

(IR-07.3) Tissue Subtype Identification using Photothermal Mid-infrared Spectroscopic Imaging

Chalapathi Gajjala¹, Rupali Mankar¹, Ragib Ishrak¹, Xinyu Wu¹, Reza Reihani¹, Sharmin Afrose¹, David Mayerich¹, Rohith Reddy¹; ¹*University of Houston*

This work uses photothermal mid-infrared spectroscopic imaging for tissue subtype identification in gynecologic tissue samples

Vibrational spectroscopy enables biochemical identification in tissue sections. Biomedical samples such as cancerous tissue are chemically heterogeneous, and bulk spectroscopy is often inadequate to ascertain the disease state in these samples. Mid-infrared spectroscopic imaging (MIRSI) is a class of technologies that combines the molecular specificity of vibrational spectroscopy with the spatial detail provided by microscopy. Traditionally, MIRSI has been performed using Fourier transform infrared (FT-IR) imaging instrumentation. The combination of machine learning and MIRSI has facilitated the identification of tissue sub-type and cancer grades in a label-free and quantitative manner. Innovations in Quantum Cascade Lasers (QCLs) have revolutionized MIRSI, and new techniques such as discrete frequency infrared (DFIR) and photothermal IR imaging have emerged recently. These technologies are more flexible, provide higher resolution, and have essential advantages over FT-IR. We will present a comparative analysis of these MIRSI technologies in the context of biomedical imaging and discuss the benefits of each technology.

Ovarian cancer is one of the deadliest cancers among women in the U.S., with over 22,000 women diagnosed with the disease every year. Early diagnosis of the disease is essential for improving survival. To automate the process of disease diagnosis, we perform MIRSI

imaging followed by machine learning. However, this requires data of higher quality and resolution. We use the super-resolution capabilities of optical photothermal infrared imaging (O-PTIR) to analyze ovarian tissue and perform tissue subtype segmentation. Bone disorders such as osteosclerosis have spectroscopic signatures identified using MIRSI. We present imaging data and results of high-resolution MIRSI of bone samples. We also present the first study that uses polarization MIRSI to demonstrate the ability to spectroscopically identify thin collagen fibers ($\approx 1\mu\text{m}$ diameter) and their orientations, which is critical for accurate grading of human bone marrow fibrosis.

(IR-07.4) Mid-Infrared Biomarkers of Lupus Nephritis Using Optical-Photothermal imaging

Chalapathi Gajjala¹, Rohith Reddy¹; ¹*University of Houston*

Identification of statistically significant mid-IR biomarkers between wild-type mice and mice with lupus nephritis.

Mid-infrared spectroscopic imaging (MIRSI) combines the molecular sensitivity of IR spectroscopy with the spatial specificity of microscopy. Fourier Transform Infrared (FTIR) imaging, the traditional MIRSI technology, has been used in varied applications, including archeology, material characterization, and cancer grading. However, the spatial resolution of FTIR is limited due to the long mid-infrared wavelengths used. Optical photothermal infrared imaging (O-PTIR) overcomes the resolution limitations of FTIR, which is especially important in specific biomedical applications such as histopathology.

Histologic assessment is the current standard of care for diagnosing lupus nephritis (LN). However, the qualitative nature of LN diagnosis and poor inter-pathologist concordance worsen disease outcomes. Despite being crucial, the markers derived from traditional immunohistochemical staining do not provide a complete picture of the disease heterogeneity and phenotype. MIRSI provides both biochemical and morphological information without stains and is a promising technique to improve the diagnostic efficacy of LN. MIRSI can quantitatively measure the changes in morphology and distribution of the kidney's intrinsic biomolecular constituents, such as collagen, lipids, and nucleic acids, contributing independent information to histopathologic assessment. In our current work, we use a murine model to assess the diagnostic efficacy of O-PTIR imaging by comparing spectral differences between Wild-Type (WT) and LN mice. We identify and present several characteristic mid-IR spectral biomarkers that demonstrate statistically significant differences ($P < 0.001$) between WT and LN mice

(IR-07.5) High-Speed Photothermal Mid-Infrared Spectroscopic Imaging Through Optimization of Sampling Parameters

Rupali Mankar¹, Rohith Reddy¹, Chalapathi Gajjala¹, David Mayerich¹, Xinyu Yu¹;
¹*University of Houston*

Proposed high-speed imaging with OPTIR overcomes long imaging limitation and enables large data collection.

Mid-infrared spectroscopic imaging (MIRSI) provides spatially-resolved molecular data by measuring the absorbance spectrum at every pixel in an image. The spatial resolution of MIRSI using Fourier Transform Infrared (FTIR) imaging is fundamentally diffraction limited. Imaging biomedical samples often require spectral interrogation in the fingerprint region (800 to 1700 cm^{-1}), and the corresponding resolution is limited to 5 μm to 10 μm . Optical-photothermal IR imaging (O-PTIR) overcomes this limit using a pump-probe mechanism and enables MIRSI at a spatial resolution of 0.5 μm . However, higher spatial resolution comes at the cost of higher imaging time, which is a significant challenge in practical MIRSI imaging of large biological tissue samples. Optimizing sampling parameters using compressive sensing algorithms can reduce data collection time while maintaining higher spatial resolution. This study presents an optimized imaging and image processing pipeline to enable high-speed O-PTIR data collection.

We optimize sampling parameters based on the sparse reconstruction of O-PTIR data and speed up the imaging time to 10X. We enhance data quality using a curvelet-based unsupervised image fusion algorithm designed for O-PTIR imaging. This study presents an analysis of the variation in data quality as a function of imaging parameters and determines optimal imaging parameters for high-speed imaging. Our results demonstrate data at spatial resolutions comparable to clinically relevant histopathology while reducing imaging time by an order of magnitude. The fused output simultaneously provides the benefits of high spatial resolution and label-free molecular contrast. The proposed imaging pipeline enables large-scale data collection on diverse tissue samples. We demonstrate the efficacy of our algorithm in ovarian cancer tissue imaging.

22LIBS03: Advanced Approaches II

Chair: Jhanis Gonzalez

(LIBS-03.1) Femtosecond LIBS Plasmas Induced by GHz Burst Mode Ablation

Vassilia Zorba¹, Minok Park¹, Xianglei Mao¹, Costas Grigoropoulos², Vassilia Zorba¹;
¹*Lawrence Berkeley National Laboratory*, ²*UC Berkeley*

GHz bursts of femtosecond (fs) pulses have emerged recently in the field of laser processing as a potential source for a new phenomenon termed “cold ablation”. Ultrafast bursts of pulses of GHz repetition rate (i.e., pulses separated by times of the order of 1 ns) have yielded enhancements of removal rates in different target materials. However, the exact mechanisms for this new phenomenon, the ablation dynamics and the utility of this technology for LIBS applications remain unknown to date,.

In this work we study the expansion dynamics of laser-induced plasmas produced by GHz bursts of femtosecond laser pulses and explore the mechanisms of the process as the basis for application in high precision, controlled spatial and depth resolution laser sampling for LIBS. Specifically, we use both laser scattering and direct plasma plume emission imaging at different femtosecond laser energy regimes to elucidate these processes for their use in LIBS analysis. Additionally, we obtain complementary information about the laser matter interaction by analyzing the laser-induced craters obtained with single fs and GHz bursts of fs pulses with white-light interferometry and scanning electron microscopy, to unveil fundamentally different laser ablation removal rates and crater topologies. This work provides new insights into complex phenomena dominating laser-matter interaction in the GHz burst regime.

(LIBS-03.2) Nuclear Safeguards with Laser-Induced Breakdown Spectroscopy

George Chan¹; ¹*Lawrence Berkeley National Laboratory*

Nuclear power is a carbon-free energy source and currently contributes to about 10% of the global electricity supplies. To ensure nuclear facilities and materials are not misused and not diverted from peaceful uses, international safeguards measures are implemented worldwide. Shipping uranium samples to an off-site laboratory for analysis is not only costly and cumbersome in logistics, but the results are not available without delay after sample collection. Developments of method and instrumentation to make field-deployable U isotopic measurements would greatly expand the toolbox available to safeguards inspectors. Optical spectroscopy (e.g., laser induced breakdown spectroscopy, LIBS) is a viable means for isotopic analysis of actinides because different isotopes emit at slightly different wavelengths (isotope shifts). LIBS is also highly adaptable for field deployment and operation because of its relatively simple instrumentation. Furthermore, isotopic emissions from ²³⁵U and ²³⁸U are measured simultaneously, which eliminate correlated noise from the laser induced plasma. Isotopic information of the sample can be extracted from the acquired spectrum with theoretical multi-variable non-linear spectral fitting, which involves no use of calibration standards. We have recently optimized the spectral window for U enrichment assay. In this presentation, our efforts on developing LIBS as a next-generation tool for nuclear safeguards will be described and discussed.

(LIBS-03.3) Quantitative Evaluation of U-Zr Alloy Fuels Utilizing Femtosecond LIBS

Matthew M. Jones¹, Matthew M. Jones¹, Joey Charboneau, Nick Erfurth, Laura Sudderth;
¹*INL*

Quantitative analysis of nuclear fuel prior to and after irradiation can be an expensive and time-consuming endeavor due to the unavoidable processes necessary to gather accurate and reproducible results. An analysis can take anywhere from a few days to multiple weeks depending on the number of samples, complexity of the fuel type, and measurement requests. Customary methods involve dissolving the fuel with inorganic acids, preparing various standards/dilutions for each instrument, performing separations to remove interferences, executing quantitative measurements, and reviewing data. Furthermore, the instruments only consume a few milliliters of sample, adding to the overall cost since

hundreds of milliliters of unused radioactive liquid must be disposed of under stringent waste requirements. These factors prompt the need for an updated approach that improves upon the pitfalls of traditional methods while making notable advances in radiochemical analysis. Femtosecond Laser Induced Breakdown Spectroscopy (fs-LIBS) offers many advantages such as: increased throughput due to minimal sample preparation/disposition, rapid elemental analysis independent of material type, and spatial resolution at the micron (1-100 μm) level. However, nuclear-based LIBS has historically been limited to a qualitative technique due to lack of suitable standards needed to produce quantifiable results. This presentation will describe potential pathways for quantitative LIBS analysis of nuclear samples through the fabrication of matrix-matched standards. Specifically, this talk will focus on the univariate calibration of U-xZr ($x = \text{wt.}\%$) binary metal alloys ranging from U-6Zr up to U-56Zr and subsequent quantification of a commonly utilized nuclear fuel type in U-10Zr.

(LIBS-03.4) The Application of TOF-ICP-MS to Life Sciences. The Advantage of a Broader Element Coverage and Faster Data Acquisition Times in the Field of Metallomics.

Lukas Schlatt¹, Phil Shaw¹; *¹Nu Instruments*

Fast accurate full elemental data analysis of metals in biological samples

Metals play a major role in the wellbeing of various forms of life and can be an indicator for certain types of diseases. Therefore, their role in living organisms needs to be examined precisely. Multiple approaches including single cell ICP-MS analysis and laser ablation imaging can be used to gain insights into the concentrations and localisations of elements, but the type of detector is crucial to gain the best possible data. In both these application cases, fast multielement data is ideally required and many detection systems fall short in these areas.

The Vitesse, a TOF-ICP-MS, has the capability to detect the full mass range in sub-millisecond time frames. When coupling this instrument to modern laser ablation systems this allows for the examination of almost all elements and recording large, high-resolution images in a matter of only a few hours. On the other hand, when examining single cells in solution, an exact identification of cell events can be made due to the fast acquisition times, leading to full elemental information on individual cells.

In this presentation data will be shown for a range of biological samples and single cell solutions which highlight the power that TOF-ICP-MS can deliver for these types of applications.

(LIBS-03.5) End Point Detection in Laser Machining using LIBS Emission Real Time Monitoring

Burak E. Sancaktar¹, Eduardo A. Rojas-Nastrucci¹, Susan D. Allen¹; ¹*Embry Riddle Aeronautical University*

LIBS emission measurement for real time process control of laser micromachined circuits and structures.

Recently, additive manufacturing (AM) has been used to achieve flexible, high-performance electronics and structures with tailored mechanical, electrical and optical properties, e.g., RF circuits, antennas, sensors, and metamaterials. Widely used laser trimming of AM structures is not currently amenable to real-time process control, relying on recipes that do not account for variations of material properties and geometries. We have demonstrated that real-time measurement of laser ablation can be achieved by monitoring the spectrum of the laser plasma during processing. This is the first example that we are aware of using laser induced breakdown spectroscopy (LIBS) to control laser processing of microwave materials and structures.

As an initial example, we used a fs nScript (model3Dn) pulsed laser micromachining (PLM) tool to remove a copper (Cu) layer from a dielectric substrate (Rogers R04003C), electrically isolating the Cu pad. Because ablated material tends to fall back into the PLMed trench, it is difficult to estimate when electrical isolation has been achieved using an empirical, recipe-type approach. The emission resulting from the PLM process was monitored using a fiber optic coupled compact spectrometer (StellarNet Black-Comet C-SR-14) Emission lines in the 200, 300 and 500 nm region were monitored as a function of the number of laser scans. Note that, under our PLM conditions, the 500 nm peaks are of the same magnitude as the 200 and 300 nm peaks. Emission intensity in each band follows the same pattern: initially slightly increasing, then flat, at some point sharply decreasing, and eventually returning to a baseline value; corresponding to oxidation and contamination removal, initial Cu removal, decreasing Cu and complete removal respectively. Four overlapping single laser scans achieved complete Cu removal as measured by the LIBS emission signal and the desired electrical isolation.

The LIBS emission signals can be used to control the LPM parameters in order to ensure complete removal of Cu, as in this example, without over-machining into the dielectric substrate. Other material combinations can be similarly monitored and the results fed back to the LPM system to control the LPM process.

22MASS03: Elemental and Isotopic Tracers: Technology and Applications

Chair: Kaveh Jorabchi

(MASS-03.1) Structurally Specific Mass Distribution-Based Isotopic Shifts in High-Resolution Cyclic Ion Mobility Separations Coupled to Mass Spectrometry

Gabe Nagy¹, David L. Williamson¹; ¹*University of Utah*

With the advancement of ion mobility spectrometry-mass spectrometry (IMS-MS)-based platforms to achieve higher resolution, new insights into the structure of unknown species can be enabled. Every ion analyzed has a characteristic isotopic envelope in its mass spectrum that can be directly linked back to its structural composition. Herein, the use of high-resolution cyclic ion mobility separations coupled to mass spectrometry (cIMS-MS) to probe the separation of the naturally-occurring isotopologues for isomeric analytes is presented. Not only were the isotopologues resolved from one another at extended pathlengths on the order of 50 meters, but diagnostic relative arrival times were also present that were isomeric specific in nature. Notably, functional group positioning revealed characteristic mobility shifts based on the mass distribution for each respective isomer's isotopic envelope. We anticipate that the use of such mass distribution-based isotopic shifts in high resolution cIMS-MS-based separations will enable the better identification of unknown species, particularly for glycomics and metabolomics-based applications.

(MASS-03.2) The IROA Protocol for Improving Metabolomics Data Quality

Chris Beecher¹; ¹*IROA Technologies*

One of the most important aspects of IROA is that it provides a mechanism to correct for ionization efficiency variances. These variances include ion suppression and in-source fragmentation, and variances due to changes within the source itself (in-source variances). As part of the ion suppression correction the percent of suppression may be corrected on a per compound basis. In our experiments more than 50% of the peaks are demonstrating greater than 25% ion suppression, with many compounds showing suppression rates of 60 to 90+ percent. At greater than 60% suppression the peak height will become completely uncorrelated to concentration, and at > 80% suppression the peaks are negatively correlated. Unsurprisingly, the corrected data allows the more accurate comparison of the concentration of compounds in analytical samples.

The variance corrected data may furthermore be used in a Dual-MSTUS algorithm for sample-to-sample normalization. While this is demonstrated in an experiment where the sample sizes vary over a larger range, in normal experiments the actual size of the original sample prior to sample preparation can vary by quite a bit. The Dual-MSTUS algorithm will not only allow all of the sample in a single experiment to be normalized to one another, but will always normalize all sample to a common standard allowing for more accurate cross experiment comparisons.

The combination of these two aspects lead to significantly enhanced metabolomic datasets.

(MASS-03.3) On-Line Hyphenation of Capillary Electrophoresis with Multicollector-ICP-MS (CE/MC-ICP-MS) for Species-Specific Isotope Ratio Analysis of Sulfur Species

Björn Meermann¹, Sebastian Faßbender¹, Dariya Tukhmetova¹, Katerina Rodiouchkina², Frank Vanhaecke²; ¹*Federal Institute for Materials Research and Testing (BAM)*, ²*Ghent University*

Species-specific Isotope Ratio determination for investigation of elemental speciation processes

In many scientific fields, isotopic analysis can offer valuable information, e.g., for tracing the origin of food products, environmental contaminants, forensic and archaeological samples (provenance determination), for age determination of minerals (geochronological dating) or for elucidating chemical processes. Up to date, typically bulk analysis is aimed at measuring the isotopic composition of the entire elemental content of the sample. However, the analyte element is usually present under the form of different elemental species.^[1] Thus, separating species of interest from one another and from matrix components prior to isotope ratio measurements can provide species-specific isotopic information,^[2] which could be used for tracing the origin of environmental pollutants and elucidation of (environmental) speciation. Using on-line hyphenations of separation techniques with multicollector-ICP-MS (MC-ICP-MS) can save time and effort and enables the analysis of different species during a single measurement.

In this work, we developed an on-line hyphenation of CE with multicollector-ICP-MS (CE/MC-ICP-MS) for isotopic analysis of sulfur species. With this method, the isotopic composition of sulfur in sulfate originating from river water could be analyzed without sample preparation. The results were compared with data from off-line analysis of the same samples to ensure accuracy. The precision of the results of the on-line measurements was high enough to distinguish the rivers from one another by the isotopic signature of the river water sulfate.^[3] Next to environmental applications, a current field is species-specific isotopic analysis of biomolecules, as sulfur is the only covalently bound constituent of proteins which can be analyzed by MC-ICP-MS. Data analysis of transient signals in terms of isotope ratio determination is further issue - we developed a small free accessible App allowing for fast data analysis taking relevant aspects (e.g., mass bias correction, peak picking, ...) into account.^[4]

- [1] P. Rodriguez-Gonzalez, V.N. Epov, et al., *Mass Spectrom. Rev.* **2012**, *31*, 504-521.
- [2] V.N. Epov, S. Berail, et al., *Anal. Chem.* **2010**, *82*, 5652-5662.
- [3] S. Faßbender, K. Rodiouchkina, F. Vanhaecke, B. Meermann, *Anal. Bioanal. Chem.* **2020**, *412*, 5637-5646.
- [4] D. Tukhmetova, J. Lisek, J. Vogl, B. Meermann, *J. Anal. At. Spectrom.* **2022**, manuscript submitted for publication.

(MASS-03.4) **HPLC-Parallel Accelerator and Molecular Mass Spectrometry Analysis of ¹⁴C-Labeled Amino Acids**

David Baliu-Rodriguez¹, Ted J. Ognibene¹, Benjamin J. Stewart¹, Bruce A. Buchholz¹;
¹*Lawrence Livermore National Laboratory*

Combine extreme quantitative sensitivity of accelerator mass spectrometry with structural identification by molecular mass spectrometry.

Accelerator mass spectrometry (AMS) is the method of choice for quantitation of low amounts of ¹⁴C-labeled biomolecules. Despite exquisite sensitivity, an important limitation of AMS is its inability to provide structural information about the analyte. For some experiments this limitation is not critical since the labeled compounds are well-characterized prior to AMS analysis. However, analyte identity is important in other experiments such as metabolism or biomarker studies where the structures of its metabolites and conjugates are not known. We previously developed a moving wire interface that enables direct AMS measurement of liquid sample in the form of discrete drops or HPLC eluent without the need for individual fraction collection, termed liquid sample-AMS (LS-AMS). We placed a flow splitter immediately after the HPLC to couple the LS-AMS with a molecular mass spectrometer, providing parallel accelerator and molecular mass spectrometry (PAMMS) detection of analytes separated by liquid chromatography. The repeatability of the method was examined, and relative standard deviations of the analyte peak areas were ≤ 10.6% after applying a normalization factor based on a ¹⁴C standard. Experiments also showed that PAMMS is more reproducible when analyzing samples with ¹⁴C/C > 1.2x10⁻¹¹, 10x the natural concentration of ¹⁴C/C. Five ¹⁴C-labeled amino acids were separated and detected to provide simultaneous quantitative AMS and structural MS data, and AMS results were compared with solid sample-AMS (SS-AMS) data using Bland-Altman plots. To show potential applications of the workflow, yeast cells were grown in a medium with ¹⁴C-labeled tryptophan. Polar metabolites of the cells were extracted and analyzed by PAMMS, and two ¹⁴C peaks were detected. The peaks were identified as tryptophan and its metabolite kynurenine using high resolution molecular MS, demonstrating the ability of PAMMS to trace metabolic pathways by alignment of chromatographic, molecular MS, and AMS data. Prepared by LLNL under Contract DE-AC52-07NA27344. LLNL-ABS-835240.

(MASS-03.5) Rapid Metabolite Quantitation by Simultaneous F and Cl Speciation
Kaveh Jorabchi¹, Frenio Redeker¹, Grace Hahm¹; ¹*Georgetown University*

New ionization method enables simultaneous detection of F and Cl for quantitation with generic standards

Identification of bio-transformation products such as drug metabolites is advanced significantly by mass spectrometric techniques using soft ionization. However, lack of standards for these products make quantitation a difficult problem. Often, radio-labeling is utilized for quantitation which carries significant hurdles in synthesis of labeled parent

compounds as well as sensitivity of detection. Here, we present an elemental speciation strategy for rapid quantitation of metabolites of drugs containing F and Cl in their structures. To circumvent analytical problems for elemental ionization of F and Cl, we utilize a new ionization approach where an inductively coupled plasma is paired with nanospray and BaF^+ and BaCl^+ are quantitatively generated from fluorinated and chlorinated compounds via post-plasma ion-neutral reactions. We characterize the effect of solvent gradient on ion generation and present a single-point response factor determination strategy where compounds are quantified using one calibration LC run containing generic standards.

22PAT06: Process Analytical in Petroleum and Refinery Industries

Chair: Toni Miao

(PAT-06.1) Optimizing Spectroscopy Performance

Brian G. Rohrback¹; ¹*Infometrix, Inc.*

Optical spectroscopy is a workhorse, but to attain full benefit from the technology, we must reduce the effort devoted to producing, maintaining, and stabilizing optical spectroscopy performance in routine quality assessment. There simply is not enough manpower available to stay on top of the calibration process, making this activity ripe for automation. Over a nine-year period, a consortium has examined an unprecedented historical collection of spectra from multiple spectrometers and sixteen oil refineries. The goal was to automate the development of chemometrics models and maintain them quickly and easily. The solution is non-disruptive, fully utilizes any legacy system, and lessens the workload rather than piling on additional tasks. Robust, reliable, and timely calibrations are the result.

(PAT-06.2) Qualitative and Quantitative Analysis of Total Petroleum Hydrocarbons (TPHs) in Soil by Handheld Near-Infrared (NIR) Spectroscopy

Heinz Wilhelm Siesler¹, Toni Miao², Natasha Sihota, Frank Pfeifer, Cory McDaniel, Marina de Gea Neves; ¹*University of Duisburg-Essen*, ²*Chevron*

For the success of soil remediation and hydrocarbon exploration operations, the determination of the total petroleum hydrocarbon (TPH) content in site-specific soils is an indispensable process step. The present communication reports on the performance of a handheld Fourier-transform near-infrared (FT-NIR) spectrometer for the rapid quantitative determination of TPH in soils of two different sites by diffuse reflection measurements. For a fast go/no-go decision in exploration work or assessment of the required cleanup progress in remediation projects, a rapid - preferably on-site - determination of the content, is mandatory. Diffuse-reflection NIR spectra were recorded with a handheld NIR scanner from soil samples of two different sites with TPH reference values ranging between 350 - 30000 ppm as determined by gas chromatography and flame ionization detection with hydrocarbon fingerprinting $\text{C}_1 - \text{C}_{44}$. The spectra were used to develop partial least squares (PLS) calibration models with root mean square calibration/cross-validation errors

(RMSEC/RMSECV) ranging between 800 and 1000 ppm TPH and absolute prediction errors of 300 and 500 ppm TPH, respectively, for test samples of the two sites.

This study confirms the capability of the next generation, ultra-compact handheld FT-NIR spectrometer for predicting very low TPH contents in different soil types by soil-specific calibrations and therefore offers the potential to become a rapid on-site and in-the-field screening tool for this purpose.

(PAT-06.3) **Robust Fiber Optic Probes for Industrial Process Control**

Tatiana Sakharova¹, Viacheslav Artyushenko¹, Toni Miao², Alexey Bocharnikov¹, Alexander Novikov³, Iskander Usenov¹, Steven Barnett⁴; ¹*art photonics GmbH*, ²*Chevron*, ³*Technical University Berlin*, ⁴*Barnett Technical Services*

Fast growing demands for remote control of industrial process parameters includes the need to develop very robust fiber probes coupled with various types of spectrometers and capable to withstand harsh parameters of chemical reactions in many industrial applications: high or low temperature, high pressure, vibrations, toxic or aggressive media, electromagnetic fields, etc.

Fourier-infrared (FTIR) spectrometry in mid-IR range as well as Near-IR spectrometry are already used for chemical analysis of process *in-line* in different applications for chemical, petrochemical, food and pharma industries. Coupling of robust fiber optic probes with IR-spectrometers enables to make remote control of process parameters *in-citu*, in real time and even multiplexed to monitor several points either inside the pipe or in reactor – which is impossible to do with common analysis of media samples to be done in lab from time to time with bench spectrometers.

We present design, fabrication, and characterization of the family of Near-IR and mid-IR fiber optic probes to measure ATR, Transflection and Reflection spectra in a broad 0.3-16 μ m spectral, temperature, and pressure range. Our progress in the unique extrusion technology of polycrystalline optical PIR-fibers (Polycrystalline InfraRed) for mid-IR range 3-16 μ m allows to develop and manufacture a variety of ATR probes with very robust design - such as gas cooled High Temperature probes, Sterilizable Probes, Easy Cleanable Probes and other versions which can match customer applications.

Broad family of fiber optic probes are also produced for Near-IR, Raman and Fluorescence spectrometers and can combine 2 or more methods in the same probe shaft – to enable more accurate tracking of process parameters in broad application span today from microreactor and bio-applications to robust industrial probes.

The review comprises also the test results of operational characteristics and questions related to the probes aging under 24x7 exploitation conditions.

(PAT-06.4) **Process Gas Analysis by 785-nm Raman Spectroscopy**

Mark S. Kemper¹; ¹*Tornado Spectral Systems*

Composition of gas phase streams is of great value in refineries and petroleum processes. Using Raman Spectrometry, online, real-time measurements of gas phase streams can be performed. Here, measurement of Hydrogen (H₂), Nitrogen (N₂), Oxygen (O₂), Carbon Dioxide (CO₂), and Methane (CH₄) are shown across a range of pressures and compositions, with Limits of Detection and measurement error. Measurement of these gases is useful for monitoring and controlling input streams, output streams, and reactions, in a wide variety of processes. Using Raman Spectroscopy allows for simultaneous online measurement of these analytes.

(PAT-06.5) Near Infrared Analyzers Applied to Process Control and Optimization in the Refinery: Measurement of Light Hydrocarbon to Heavy Hydrocarbon Liquid Streams

Allan J. Rilling¹, Edward A. Orr², Jose Quintero-Escorcia²; ¹*ABB Inc*, ²*ABB Inc*.

NIR measurement in refinery extended from light hydrocarbon streams to heavy liquid hydrocarbon streams

Optical spectroscopy utilizing near infrared absorption measurement has been applied extensively and successfully in the refinery for many years. Vibrational spectroscopy allows for fast and high-quality measurement of liquid hydrocarbon streams that allow a means to provide rapid and high-quality monitoring and feedback to achieve process optimization. FT-NIR (Fourier Transform – near infrared) analyzers) have demonstrated many years of effective measurement of light hydrocarbons streams such as gasoline, kerosene, naphtha, diesel, etc, as well as applied across a range of process units within the refinery (from CDU, CCR, NSC, HFU to final product blend). Refineries seek every means to increase margins which requires optimizing refinery process unit operations for light to heavy streams. The same base technology also allows measurement for heavier viscous hydrocarbon streams extending to lube oils and crude where some optical techniques may be limited. The challenges for heavier over lighter streams are not insignificant due to viscous sample properties and modelling required. An overview of online measurement in the refinery will be presented covering light to heavy Streams in the refinery.

22PMA06: Advanced Spectroscopic Techniques in PAT Part I

Chair: John Wasylyk

Co-Chair: Mike George

(PMA-06.1) Coherent Control of Chemical Reactions Using Floquet States

John C. Wright¹, Kent J. Meyer², Lucian Hand³, Martynas J. Miškinis³, Vytautas Sinkus³, Nick Adams³; ¹*University Wisconsin-Madison*, ²*UW Madison*, ³*Light Conversion USA*

A new family of Floquet state spectroscopies coherently control reactions and characterize reaction mechanisms.

The advent of lasers brought with it the promise of using coherence to drive chemical reactions. Theoretical and experimental work focused on shaping femtosecond laser pulses using genetic learning algorithms for feedback control of pulse shapes that optimized reactions. This talk describes a different approach that has successfully implemented coherent reaction control using a Floquet state methodology. A Floquet state is a periodically driven and entangled quantum system containing multiple states simultaneously. The state remains coherent during the entire time it is driven and since they are coupled to each other, their nature is fundamentally changed from the equilibrium ground state. By entangling the fundamental, overtone, and combination band vibrational states that form the coordinates of a reaction potential energy surface, it becomes possible to create and maintain the same vibrational states that are created by the chemical reaction potential energy surface for thermally driven reactions. Controlling the driving frequencies near resonances allows direct manipulation of the coupling and control of the reaction. This talk will describe an extensive experimental demonstration of coherent control of metal carbonyl decomposition chemistry by Floquet states consisting of up to 12 vibrational states and the determination of the multidimensional potential energy surface of the decomposition.

(PMA-06.2) Fiber Spectroscopy for in-line Process Control in 0.3-16 μ m Range

Viacheslav Artyushenko¹, Viacheslav Artyushenko¹; ¹*art photonics GmbH*

Fiber coupled multi-spectral systems and customized sensors used for process-control in-line in 0.3-16 μ m range

Advanced fiber probes based on different fiber types will be presented for their coupling with 4 different spectrometers used in broad range of spectra 0.3-16 μ m. This innovative multispectral fiber system enables to test all key spectroscopy methods: Transmission, ATR-absorption, Raman, fluorescence and their various combinations for reaction monitoring *in-line* - when all 4 fiber probes immersed in the same reactor.

This possibility allows to compare all methods and to enhance accuracy, specificity and sensitivity of process-control *in-line* (or medical diagnostics *in-vivo* for tumor margin detection) by 2 ways:

- a) to select the most sensitive and accurate method from 4 - to provide industrial process-control for its automatization;
- b) to develop customized spectral sensor for selected process based on few spectral bands -

with substantial reduction of their size & cost compared to process--spectrometers. Such a sensors can be fabricated with IP-address each and with their data transfer to iCloud for chemometric treatment in real time. This concept will lead to the chance of creation of Industrial IoT networks based on spectral fiber sensors.

Examples of probes for multispectral analysis will be described – using Silica fibers with metal coating for 0.3-2,2 μ m range, Polycrystalline fibers for 3-16 μ m range, Hollow Waveguides for selected Mid IR-fiber windows and Chalcogenide As-S-glass fibers.

The great synergy effect in fusion of spectral data from 2 (or more) spectral methods is available now when the **advanced combi-fiber probes** collect spectra from the same spot: Raman+DRS (Diffuse Reflection Scattering), Raman+Fluorescence, Near+Mid IR-absorption, Fluorescence + Mid ATR-absorption. Advantages of combi-probe enhanced accuracy to be presented, including the demo of the smallest diameter Raman/Fluorescence probe with < 200 μ m diameter – used for a fast diagnostics of tumor margins.

The new generation of multiwavelength spectral sensors will be shown where the bundle of thin Mid IR-fibers combines radiation from the set of QCL into the innovative arthroscopy probe with side ATR-distal tip – used for in-vivo diagnostics of osteoarthritis in cartilage.

(PMA-06.3) Upstream Process Monitoring by Time-gated Raman Spectroscopy
Amuthachelvi Daniel¹, Mari Tenhunen¹; ¹*Timegate Instruments Ltd*

Online monitoring and understanding of a bioprocess hold the key for successful bioprocess development. Further the Process Analytical Technology (PAT) initiated by US Food and Drug Administration (FDA) also accentuates on the importance of monitoring the Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs). This monitoring would aid in controlling the bioprocess, reducing the number of process failures. In the current scenario, typically only pH, temperature and dissolved oxygen are monitored in real time. It would be preferred to monitor other important parameters such as glucose, lactate, ammonia, glutamine, glutamate, and lactate dehydrogenase (LDH), and the key performance indicators (KPIs) like total cell density (TCD) and viable cell density (VCD) in real time. Spectroscopic methods such as Raman spectroscopy has been actively explored for the 40 years to cater to this unmet need of the biopharmaceutical industry. Time-gated Raman spectroscopy has surpassed this impediment by collecting the Raman scattered photons before the advent of fluorescence. Chinese Hamster Ovary (CHO) cell line in a 50 l bioreactor by time-gated Raman technology was followed in real time, in-line and in this presentation we present these groundbreaking results.

(PMA-06.4) Quantum Cascade Laser (QCL)-based IR Liquid Analyzer for Real-Time Measurement of Protein Concentrations and Higher Order structures (HOS)

Jeremy Rowlette¹, Santosh Hodawadekar¹; ¹*DRS Daylight Solutions*

Quantum Cascade Laser (QCL)-based IR Liquid Analyzer for Real-Time Measurement of Protein Concentrations and Higher Order structures (HOS)

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Introduction:

There is a growing interest in adopting Process Analytical Technologies (PAT)-based technologies in the biopharmaceutical industry. Understanding Higher Order Structures (HOS) of biotherapeutics is key to minimizing protein aggregation; since aggregation impacts the secondary structure of protein molecules. Our patented QCL-IR analyzer, Culpeo, allowed us to understand how secondary structures are related to HOS. This work focuses on the case studies explaining how our QCL Mid-IR liquid analyzer helps predict real-time higher-order structures and mitigate protein aggregates.

Here we present precision-based high protein concentration measurements and secondary structure prediction in real-time using a Culpeo liquid analyzer. This unique analytical tool can serve as an orthogonal method for characterizing HOS and aggregation states at the key unit operations steps by quantifying the changes in the secondary structures of drug substances. We will also discuss the path for using this technology for at-line product identification and process fingerprinting.

(PMA-06.5) A Micro-Raman Study of Structural Changes Produced in Antimony and Antimony Chalcogenide Thin Photovoltaic films as a Result of Argon Ion Sputtering During X-ray Photoelectron Spectroscopy Experiments

Tariq Jawhari¹, Xavier Alcobé¹, Lorenzo Calvo-Barrio¹, Diego Fraga Chiva², Samuel Porcar García², Juan Bautista Carda Castelló², Isidro Martin Garcia³; ¹*CCiT, Universitat de Barcelona (UB)*, ²*Universitat Jaume I*, ³*UPC*

Unusual crystallization effects produced by sputtering processes in amorphous antimony layers detected by Raman microscopy

X-ray Photoelectron Spectroscopy (XPS) or ESCA (Electron Spectroscopy for Chemical Analysis) is a powerful elemental tool for the characterization of material surfaces. During the XPS experiment, a slight sputtering process with argon ions of low energy (about 4 KeV) is often carried out, previously to the measurement, to remove contaminants (such as adventitious carbon) along the first few nanometers (1-2 nm) of the surface. In a preliminary study on the synthesis and development of new antimony chalcogenide thin-films by chemical routes for photovoltaic solar cells applications, several analytical techniques were

used to characterize these materials, including XPS, Raman spectroscopy and X-ray Diffraction (XRD). It was initially observed that the XPS experiment may produce, in some cases, some structural changes in some of the films. In the present work, several antimony and antimony chalcogenide layers (Sb; Sb₂S₃; Sb₂Se₃) were submitted to an argon ion sputtering process during the XPS measurements, varying the experimental conditions, such as the sputtering time, x-rays irradiation, etc. These irradiated samples were characterized by micro-Raman spectroscopy and XRD. These two analytical techniques clearly indicate that an unusual crystallization process takes place in some of the amorphous layers. Raman microscopy also allowed the detection and identification of some unusual micro-morphological structures that were observed by optical microscopy in some of the samples submitted to a sputtering process. These variations in the morphology were correlated to microstructural changes in the films. Some other materials such as amorphous silicon were also studied here. In conclusion, this study shows that the singular crystallization process observed in some of the amorphous thin films analyzed here is due to argon ion sputtering that takes place during the XPS measurements.

22RAM06: Biomedical Raman (Clirspec)

Chair: Nicholas Stone

(RAM-06.1) Autofluorescence-Raman Analysis of Surgical Margins During Mohs Micrographic Surgery: Clinical Integration and Preliminary Validation Results

Ioan Notingher¹; ¹*University of Nottingham*

Mohs micrographic surgery is an advanced treatment for high-risk non-melanoma skin cancers, in which thin sequential layers of tissue are removed and checked by frozen section histology until the entire tumor is excised. While very effective, Mohs surgery is resource intensive (require dedicate histology laboratory) and requires specialist surgeons. Therefore, currently it is not widely available for a large percentage of the population. We will present the development of a new technique based on Raman spectroscopy and auto-fluorescence imaging, developed as a potential alternative to frozen section histology. The key advantages of the technology is that skin layers of skin can be analysed directly after excision without requiring sectioning or staining. The instrument was designed to scan the entire resection margin within 30 minutes and provide a pseudo-colour coded image that identifies the residual tumour based on quantitative machine learning analysis of the Raman spectra. The instrument was integrated into clinical practice, and preliminary measurements have been performed to assess its performance (115 patients). The presentation will include key aspects related to the technology the preliminary estimates on diagnosis accuracy.

(RAM-06.2) Deep Raman Spectroscopy: Multiplexed Signal Recovery for Future Theranostics

Ben Gardner¹, Nicholas Stone¹, Pavel Matousek², Sara Mosca³, Francesca Palombo¹, Megha Mehta, Marzieh Salimi¹; ¹*University of Exeter*, ²*STFC Rutherford Appleton Laboratory*, ³*CLF, RAL, STFC*

The Deep Raman Spectroscopy (DRS) techniques i.e. Transmission Raman and Spatially Offset Raman Spectroscopy (SORS), are poised to have a transformative role in many aspects of healthcare from diagnosis, surgical margin analysis, disease monitoring, treatment and all of the above in one, as future theranostics. For this transformative role it is important that the technique can demonstrate its capabilities to multiplex key information: 1) where you are sensing, 2) what you are sensing, 3) changing the local environment i.e. heating. All of this needs to be accomplished in near real-time, with high accuracy and when possible in a robust quantitative fashion. Here we demonstrate the development of optically controlled phantoms with complex geometries to better simulate *in vivo* targets, and replicate expected analytical complexities while utilising surface enhanced Raman spectroscopy to probe quantitatively and change the local environment in a multiplexed way in real-time.

(RAM-06.3) Non-Invasive Multimodal Spectroscopic Diagnosis for Early Stage Oral Cancer

Siddra Maryam¹, Daniyal Ghauri¹, Rekha Gautam¹, Kiang Kho¹, Marcelo S. Nogueira¹, Sanathana k. Sekar¹, Huihui Lu¹, Richeal Riordain², Linda Feeley³, Patrick Sheahan⁴, Ray Burke¹, Stefan Andersson-Engels¹; ¹*Tyndall National Institute*, ²*Cork University Dental School and Hospital*, ³*University College Cork*, ⁴*Cork Ireland South Infirmary Victoria University Hospital*

Identification of spectral biomarkers involved in oral carcinogenesis utilizing a non-invasive multimodal spectroscopic system.

Oral cancer is one of the most common malignancies in the world. Diagnosis of oral cancer at early stage is often complex due to uncertain development of a benign lesions into cancer and the impracticality to take biopsy of every lesion. This study aims to develop a multimodal scheme for diagnosing oral cancer non-invasively in the early stages and to assess the performance of an integrated spectroscopic diagnostic platform comprising of Raman and diffuse reflectance spectroscopy. In this study, patients undergoing biopsy or histopathological examination will be recruited. For *in vivo* analysis, malignant tissues as well as contralateral site will be examined using a fiber-optic probe to identify and integrate spectral biomarkers involved in carcinogenesis through both modalities. In parallel, *ex vivo* measurement will also be carried out on saliva specimens using surface-enhanced Raman spectroscopy (SERS). This technique aims to detect proteomic biomarkers in saliva for oral cancer detection. To improve the repeatability and sensitivity of the analysis, photonic crystal fibers (PCF) will be used instead of a flat 2-dimensional SERS substrate for saliva analysis. These fibers would provide the advantage of long light-molecule interaction length in a relatively small volume (20 - 100nL range), which in turn improves the sensitivity. This

multimodal optical diagnostic approach has potential to improve diagnostic accuracy and patient survival rate by detecting oral cancer at early stage.

(RAM-06.4) High-resolution Raman Imaging of >300 Cells from Human Patients Affected by Nine Different Leukemia Subtypes: Virtual Staining Using a Global Clustering Approach

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Here, we report the currently most extended Raman imaging-based characterization of leukemia cells from patients

Background: Leukemia defines a large group of tumors characterized by the proliferation of immature cells (“blasts”) in the bone marrow. According to specific morphological and genetic features, more than forty leukemia subtypes should be recognized to define diagnosis and treatment. Even if nowadays the definitive diagnosis is supported by molecular analyses, the visual assessment of bone marrow (BM) smears is still a fundamental step, and it is based on morphology. This process is subjective, time-consuming, and suffers from intra- and inter-observer variability. Despite various Raman-based studies aimed to classify leukemia cells, their morphological characterization and classification is missing.

Methods: BM samples from 19 patients affected by 9 different leukemias, including 6 acute myeloid leukemia (AML) subtypes (M0, 1, 2, 3, 5a and 6) and 3 acute lymphoid leukemia (ALL) subtypes (BPh+, BPh-, T) were selected. A total of 319 cells were studied by high-resolution Raman imaging using a home-built confocal Raman microscope (647 nm laser, 63xW). Each cell was scanned by 64x64 (4096) Raman spectra using ≥ 180 nm step size and 100 ms/pixel. After automatic pre-processing of the entire dataset (1) >667k spectra relative to the cellular region were retrieved and clustered (n. clusters = 17), aiming to produce pseudo hematoxylin-eosin (HE) images; (2) The resulting per-cell cluster distribution was used with classification aims and (3) averaged spectral data from each cell were used, in parallel, to automatically identify different leukemia subtypes by machine-learning approaches (i.e. random forest).

Results: the clustering approach applied to the 319 high-resolution Raman maps allowed to 1) automatically produce pseudo HE stained images and 2) to characterize leukemia subtypes and relative morphological/biochemical features according to clustering results. In parallel,

averaged spectral data processed by machine-learning approaches enabled to distinguish AML vs ALL subtypes with sensitivity of 0.93, 0.91 (leave one cell out) or 0.80, 0.73 (leave one patient out), respectively, and to identify the most important discriminating features. This study demonstrates the potential of Raman imaging and whole-dataset clustering approaches for the assessment of leukemia cells, revealing very typical biochemical profiles of specific leukemia subtypes.

(RAM-06.5) Raman Endoscope for Diagnosis of Eosinophil Esophagitis

Hidetoshi Sato¹, Riki Zakaria¹, Takumu Watanabe¹, Soichiro Ishihara¹, Keita Iwasaki¹, Bibin Andriana¹, Kosuke Hashimoto¹, Tatsuyuki Yamamoto², Naoki Oshima²; ¹*Kwansei Gakuin University*, ²*Shimane University*

Raman analysis for detection of eosinophil accumulation in live esophagus tissue

The purpose of the presents study is to develop a Raman probe for endoscope and to evaluate the viability of Raman analysis for detection of eosinophil accumulation in live esophagus tissues of a model mouse. The side-view Raman probe is developed based on the ball lens top hollow optical fiber Raman probe (BHRP) with a half-ball lens made of fused quartz. Spectra of the live tissue with blood flow measured with 785 nm-excitation light showed very weak signal due to hemoglobin (Hb). It suggested the possibility to detect eosinophil accumulation in the tissue with the 785 nm-excitation light. The excitation wavelength of 633 nm induced strong fluorescence of sapphire glass that is a material of the ball lens of BHRP. On the other hand, the previous study suggested that eosinophil including eosinophil peroxidase (EPO) that showed a strong resonance Raman effect with 633 nm-excitation light. The Raman spectra of eosinophil as well as erythrocyte and other granulocytes measured with 633 and 785 nm-excitations were compared to estimate the viability of Raman analysis for in situ diagnosis. The Raman spectra of eosinophil showed strong contribution of EPO, suggested that a heme chromophore in EPO had pre-resonance enhancement via Q band even with the 785 nm-excitation light. Principal component analysis (PCA) was applied for analysis of Raman spectra of eosinophil, erythrocyte and other granulocytes. Eosinophil was successfully discriminated from other blood cells in the PCA score plots built for the datasets of the spectra measured with 633 and 785 nm-excitation wavelengths. The Raman spectroscopy with 785 nm-excitation had high viability for in situ analysis of eosinophilic esophagitis (EoE).

22RAM10: SAS - SPECTROSCOPY IN SPACE

Chair: Andrew Whitley

(RAM-10.1) Recent Advances in Long-Range Remote Raman Systems for Planetary Exploration

Shiv K. Sharma¹, Shiv K. Sharma¹, Stanley M. Angel², Paul G. Lucey, Tayro Acosta-Maeda, Evan M. Kelly¹; ¹*University of Hawaii at Manoa*, ²*The University of South Carolina*

Raman spectroscopic instruments are finding increasing applications on rovers and landers for planetary exploration. For example, on the Mars 2020 Rover Mission, the SuperCam instrument has a time-resolved remote Raman spectrometer using a 532-nm laser as well as an in situ deep UV Raman system as a part of the SHERLOC instrument. These instruments are capable of providing information on mineralogy, mineral coatings as well as on organic matter on the surface of Mars. While remote Raman extends the range of the Perseverance rover to 7-10 meters, the successful demonstration of the Ingenuity helicopter during the ongoing Mars 2020 Mission and NASA's planned 2027 Dragonfly Mission to Saturn's Moon Titan show the need to greatly extend the range of the remote Raman instruments to much greater distances for future missions.

At the University of Hawaii (UH), we have extended the range of Raman measurements of geological materials and chemicals to greater than 100 m by direct coupling a miniature high-throughput time-resolved Raman spectrometer with a 3-inch diameter catadioptric telescope and a pulsed 532-nm laser. The miniaturized Raman spectrometer is based on a small F/2 transmission grating monochromator with a small intensified CCD (ICCD) camera. The miniature ICCD has the intensifier directly coupled to the CCD via an optical fiber plate. The miniaturized Raman instrument could be mounted on a stationary lander platform or on a rover. To further reduce the size and expand planetary applications of remote Raman spectroscopy, UH and the University of South Carolina, are investigating monolithic Spatial Heterodyne Spectrometers. Using monolithic construction techniques, the footprint of the monolithic spatial heterodyne Raman spectrometer (mSHRS) is only a few square cm. The new small robust designs allow Raman instruments to be amenable for use on future planetary landers, rovers, and helicopters. In this talk, we will describe the newly developed miniature Raman spectrometers for future planetary missions, including mounting them on planetary drones for remote Raman spectroscopy.

This work is supported by NASA and NSF grants.

(RAM-10.2) Exploring Jezero Crater with SuperCam on the Perseverance Rover

Sam Clegg¹, Ann Ollila², Ryan Anderson³, Olivier Forni⁴, Agnis Cousin⁴, Jeremie Lasue⁵, Chip Legett, Paolo Pilleri⁵, Elise Clave⁶, Shiv K. Sharma⁷, Olivier Beyssac⁸, Jeff Johnson, Guillermo Lopez Reyes, Nina Louise Lanza², Baptiste Chide², Juan Manuel Madariaga, Sylvestre Maurice⁵, Roger Wiens⁹, The SuperCam Team; ¹*Los Alamos National Lab*, ²*Los Alamos National Laboratory*, ³*US Geological Survey*, ⁴*Institut de Recherche en Astrophysique et Planétologie*, ⁵*IRAP-CNRS*, ⁶*Université de Bordeaux*, ⁷*University of Hawaii at Manoa*, ⁸*IMPMC, Paris, France*, ⁹*Purdue University*

SuperCam is the remote sensing instrument operating on the Perseverance rover. It is the integration of Laser-Induced Breakdown Spectroscopy (LIBS), 532 nm Time-Resolved Raman spectroscopy, Time-Resolved Luminescence Spectroscopy (TRLS), Visible and

Near Infrared Spectroscopy (VISIR), color Remote Micro-Imager (RMI), and a microphone. Part of SuperCam is located at the top of the rover mast where it can analyze rocks and soils from a 2 to 7 m standoff distance in any direction around the rover. The SuperCam suite of spectroscopic techniques are all co-boresighted and can be employed to determine the chemical (LIBS, TRLS) and mineralogical (Raman, VISIR, TRLS) composition of each target, depending on the scientific objective of the specific investigation. The RMI provides high resolution geologic context images of every target probed as well as spectacular long-distance images. The microphone has been used for several scientific investigations including sample physical properties from the sounds generated by the LIBS sparks.

The Perseverance rover landed in Jezero crater on February 18, 2021. Orbital observations indicate that Jezero crater (~45 km diameter; formed ~3.8 billion years ago) hosted a lake, as indicated by both inflow and outflow channels, and an ancient river delta, suggesting a potentially habitable environment. Orbital observations also suggested the presence of clays, carbonates, and olivine. SuperCam has indeed documented a rich chemical and mineralogical diversity along the rover traverse including olivine, clays, carbonates, and sulfates. This presentation will highlight some of the diverse chemical and mineralogical observations made by SuperCam as well as discuss the complementary nature of these analytical techniques to characterize the targeted samples. Finally, the development of the multivariate analysis methods used to extract quantitative chemical compositions will be discussed.

(RAM-10.3) **SHERLOC: Results of the First 400 Sols of Operations**

Luther Beegle¹, Rohit Bhartia², William Hug, SHERLOC Science Team; ¹*California Institute of Technology*, ²*Photon Systems, Inc.*

On February 18th 2021, the Perseverance rover landed in Jezero crater, Mars. This site was chosen because orbiter data analysis provides evidence that the crater hosted a stream-fed lake at a time in the Martian Noachian period. The Octavia Butler landing site is located ~1.9 km east of the remnants of a river delta. Deltaic and lacustrine sediments can preserve biosignatures, making Jezero crater a prime target for Mars sample return science. One of the seven instruments of Perseverance's science payload is SHERLOC –Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals.

SHERLOC combines fluorescence and Raman spectroscopy with microscopic imaging to analyze surface material to better understand the history of the aqueous environments recorded in the rocks of Jezero crater and to search for potential biosignatures. SHERLOC imaging consists of two microscopic cameras, the Autofocus and Context Imager (ACI), and the Wide-Angle Topographic Sensor for Operations and eNginneering, (WATSON). These subsystems obtain high spatial resolution images of geological targets to identify grain-scale structure and texture.

SHERLOC spectroscopy enables high-sensitivity detection, characterization, and spatially-resolved correlation of trace organic materials. SHERLOC's 248.6 nm deep UV laser generates a 100 µm-diameter spot. Photons generated by Raman scattering and fluorescence

emission are collected and spectra are downlinked to Earth for analysis. Knowledge of where the laser is pointed allows for mineral and compositional maps to be generated and overlain on images.

In the first 400 sols, SHERLOC has been able to identify, Phosphates, Sulfates, Carbonates and amorphous silicate in abraded patches in the green zone campaign within Jezero Crater. Within these detections we have begin to tell the story of what this crater was like when it was full of liquid water over 3 billion years ago.

(RAM-10.4) SHERLOC: Deep UV Raman from Earth to Mars

Rohit Bhartia¹, Rohit Bhartia¹, Luther Beegle²; ¹*Photon Systems, Inc.*, ²*California Institute of Technology*

Detecting organics and minerals using the combination of deep UV fluorescence and Raman mapping spectroscopy has only recently developed into laboratory instruments. However, given recent technological advancements in both laser and optics, we have been able transition this deep UV spectroscopic analysis for Mars astrobiology exploration in the search for signs of life through SHERLOC instrument, an instrument on the NASA Mars 2020 Perseverance rover. SHERLOC (Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals) is a proximity instrument operating from the end of the rover arm. It includes a wide-angle imager (WATSON) to provide color imaging and context at multiple spatial scales, and a high-resolution (10 $\mu\text{m}/\text{pixel}$) imager that is co-boresighted with the mapping deep UV fluorescence and Raman spectrometer. Combined the SHERLOC instrument enables a new method to detect and spatially resolve trace organics and minerals.

Deep UV fluorescence is highly sensitive to aromatic ring organics. Since the excitation is at 248.6 nm, simple aromatics rings are detectable as well as aromatic heterocycles and 2 ring aromatics. We previously demonstrated that the fluorescence, when excited < 250 nm, the detection of and differentiability of organics is enhanced and enables rapid detection of trace organics with picogram sensitivities. However, while sensitive, fluorescence is limited by broad spectral features. Fusing the fluorescence with deep UV Raman spectroscopy adds higher levels of differentiability and excitation

This presentation will discuss the science and engineering that went into developing SHERLOC and with some recent examples, how it operates on the surface of Mars. In addition, the future benefits and opportunities of deep UV fluorescence and Raman spectroscopy for planetary science as well as the opportunities that are now enabled for terrestrial applications.

(RAM-10.5) Panel & Open Discussion

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22SPR05: Early Career Researchers in Plasmonics

Chair: Zac Schultz

(SPR-05.1) Transforming Treatment of Patients with Drug Induced Liver Injury Using SERS Based Lateral Flow Testing

Sian Sloan-Dennison¹, Ben Clark, Kathleen Scullion, James Dear, Dieter Bingemann², Paul Fineran, David Creasey², Cicely Rathmell², Karen Faulds¹, Duncan Graham¹; ¹*The University of Strathclyde*, ²*Wasatch Photonics*

The bestselling non-prescription drug, acetaminophen, is used by millions of people worldwide as a safe and effective method of pain relief. However there are a large number of acetaminophen overdoses, both accidental and intentional. Due to the increase in toxic metabolites in the liver caused by overdose, drug induced liver injury (DILI) occurs which, if left untreated, can result in a patient requiring a liver transplant or even death. Currently, when a patient presents to hospital following an overdose, the severity of it is determined by drawing blood intravenously and sending the sample to a central lab within the hospital where the level of alanine aminotransferase (ALT), a biomarker for hepatocyte injury, is measured. Although ALT is an established clinical biomarker for detecting DILI, its level in blood following acetaminophen ingestion is slow to rise, resulting in missed or delayed DILI diagnosis. Recently a new protein biomarker, keratin-18 (K18), has become a candidate for the accurate and early detection of DILI. To maximise the benefit of K18 a rapid, quantitative detection test, which can take place next to a patient, has to be developed.

For the rapid detection of K18, a paper-based lateral flow strip combined with surface enhanced Raman scattering (SERS) analysis has been proposed. Conventionally, SERS analysis of lateral flow strips has been carried out on large Raman microscope systems by mapping the test line. This analysis can take a number of hours, uses instrumentation with no portability aspect and has to be carried out by trained personnel. An alternative, which allows measurements to be taken next to the patient, is to use a portable SERS spectrometer. By adapting point and shoot spectrometers to include accessories which can accommodate lateral flow cassettes and line illumination optics, DILI status has been successfully determined in patient serum samples rapidly and sensitively. Incorporating this platform as part of the standard care protocol would lead to quicker administration of treatment and potentially reduce the number of liver transplants and deaths associated with acetaminophen overdoses.

(SPR-05.2) Plasmon-Enhanced Electrochemistry in Nonaqueous Solvent

Andrew J. Wilson¹, Padmanabh Joshi¹; ¹*University of Louisville*

Noble metals are popular choices for electrochemical transformations due to their high catalytic activity and relative inertness. When nanostructured, noble metals also support plasmon resonances which can be leveraged to increase electrochemical activity and impart novel reaction selectivity by irradiating noble metal electrodes with light. To date, the utility of plasmonic materials as photoelectrocatalysts has been demonstrated for numerous chemical reactions in aqueous media. However, nonpolar gases and a broad range of organic molecules have limited to poor solubility in water. In this talk, I will present our studies that probe the plasmonic enhancement of a model electrochemical reaction in a

nonaqueous solvent. We use linear sweep voltammetry to measure the enhancement of the water reduction reaction in acetonitrile occurring on electrochemically roughened Au electrodes irradiated with visible light. Under light irradiation, the applied electrical bias required to drive the reaction is reduced suggesting the development of a photopotential on the plasmonic Au electrodes. The measured photopotentials are inversely proportional to the concentration of water in the acetonitrile solvent. At the lowest concentrations of water, we measure a photopotential of ca. 175 mV, an approximately 3.5× improvement over solvent conditions with a relatively high proportion of water. Electrode heating under laser irradiation was determined to have a negligible impact on the enhancement of the reaction in our experimental conditions. We propose a mechanism by which an increase in solvent polarity increasingly stabilizes photocharged plasmonic electrodes, lowering the photopotential available to enhance the electrochemical reaction. Our results demonstrate the applicability and promise of using plasmonic electrodes to enhance electrosynthesis in media beyond water.

(SPR-05.3) Exploring Chemistry of Surface-Supported Nanostructures using Ultrahigh Vacuum Tip-Enhanced Raman Spectroscopy

Sayantan Mahapatra¹, Nan Jiang¹; ¹University of Illinois Chicago

Tip-Enhanced Raman Spectroscopy is shown as an advanced spectroscopic technique to probe molecule's chemical fingerprint.

Conventional spectroscopic techniques are limited by the optical diffraction limit to about half wavelength and therefore offer about 200 nm x 200 nm microscopic zone for working in the visible light range. Tip-enhanced Raman spectroscopy (TERS) emerges as an advanced analytical technique, where the plasmonically active probe is not only used to detect the tunneling current but also to interrogate the local chemical environment of the surface adsorbed molecules with angstrom scale precision. In this work, we report a topological and chemical analysis of two regioisomers (positional isomers), trans- and cis-tetrakis(pentafluorophenyl)porphodilactone (trans- and cis-H₂F₂₀TPPDL) by scanning tunneling microscopy (STM), ultrahigh vacuum (UHV) TERS on Ag(100) with the spatial resolution down to 8 Å, which has a wide range of applications in various field of surface science & nanotechnology such as regioselective catalysis reaction, chemical reactions, molecular electronics, etc. We have shown, it is possible to distinguish these two structurally very similar forms with high accuracy & precision. The two-component molecular junction has been identified using high resolution two-dimensional (2D) Raman mapping. In addition, the molecule-substrate interactions have been addressed at the single-molecule level by employing three different single-crystals i.e., Ag(100), Cu(100), and Au(100). Strong surface interactions at Cu(100) surface converted the flexible porphodilactone structure inverted, which was further verified by STM. Angstrom scale chemical analysis using TERS is shown here as a broad and versatile technique in surface characterization. Additionally, the highly energetic plasmons generated at the tip apex can also be utilized for site-selective activation inside a single molecule which could prove extremely useful in solar-to-chemical energy conversion-related problems.

(SPR-05.4) Advances in SERS Optophysiology for Neurosciences

Stephanie M V Gallant¹, Jean-Francois Masson¹; ¹*University of Montreal*

Plasmonic nanofibers for localized Raman sensing additionally work as optogenetic probes for stimulating biological processes

Plasmonic sensors on the nanoscale are of significant interest in surface-enhanced Raman scattering (SERS) based biological sensing, as they offer powerful, fast, and label-free sensing while requiring small amounts of sample. When investigating neural tissues, our gold nanoparticle-decorated nanofibers have allowed for spatially-specific sensing in regions of interest in the brain, providing information about the neurotransmitters present within the local environment. Beyond simple sensing, these plasmonic nanofibers are also being explored as probes for optogenetic applications in-vivo, both stimulating and subsequently measuring the secretion events occurring. We are additionally optimizing these fibers for use with near-IR laser systems, of which wavelengths better suit many biological tissues of interest.

(SPR-05.5) Surface Enhanced Spatially Offset Raman Spectroscopy Using A 1064 nm Laser

Andrew R. Callander¹, Karen Faulds¹, Duncan Graham¹, Neil C. Shand²; ¹*The University of Strathclyde*, ²*The Defence Science and Technology Laboratory (DSTL)*

First report of surface enhanced spatially offset Raman using a 1064 nm laser

Spatially Offset Surface Enhanced Raman spectroscopy (SESORS) has attracted great interest for non-invasive medical diagnostics with potential in-vivo application. However, the maximum tissue penetration depth is limited due to the relatively poor transmittance of visible and NIR-I (*approx.* 650-900 nm) light through tissue, coupled with unwanted Raman and auto-fluorescent interference. It is theorised that use of longer wavelength light may offer significant advantages to address these problems. Compared to NIR-I wavelengths, 1064 nm lasers permit higher maximum laser exposures, are widely reported to penetrate tissue more efficiently than NIR-I lasers and strongly attenuate fluorescent and Raman interference. However, SESORS using wavelengths larger than 830 nm has not previously been reported due to the intrinsic weakness of Raman scattering at long wavelengths and the lack of a strong plasmon resonance from gold nanoparticles.

Here, we overcome the weakness of SERS in this region by optimising multicore shell-isolated nanoparticles (SHINs) that exhibit a super-bright SERS response when irradiated with 1064 nm light. The strong SERS response enables multiplex detection through up to 1 cm of lean porcine tissue using a 1064 nm handheld spectrometer without the use of a spatial offset, while eliminating Raman and auto-fluorescent interference. Following these

successful results we construct a custom SORS system and demonstrate 1064 nm SESORS detection of nanoparticles through tissue for the first time.

To investigate the prospects for 1064 nm SESORS, we experimentally model the SERS intensity as a function of depth, wavelength and tissue type. Our preliminary evidence shows that the longer wavelength light enables deeper detection in highly turbid tissues such as porcine fat. Moreover, our results suggest that skin, fat and lean tissue exhibit a more similar response at 1064 nm compared to 785 nm, which may eventually facilitate modelling of heterogenous tissue samples.

22SPSJ04: Frontiers of Vacuum, Far, and Deep-Ultraviolet Spectroscopy II

Chair: Igor Lednev

(SPSJ-04.1) UV Raman - A Key Technology for BioPhotonics

Jürgen Popp¹; ¹*Leibniz Institute of Photonics Technology*

Raman-based technologies have proven their great potential in the fields of life sciences and medicine and increasingly complement established techniques such as fluorescence spectroscopy or microscopy. This is due to the fact that a Raman spectrum consists of many dozens of independent parameters ranging from concentration to the three-dimensional arrangement of macromolecules and biopolymers in biological samples. Thus, the advantages of Raman spectroscopy are its unprecedented high molecular specificity and its versatility, but it suffers from its low sensitivity, which limits the detection of molecules at very low concentrations. This drawback can be overcome by using special Raman signal amplification techniques. In this context UV Raman spectroscopy where the excitation wavelength is chosen to match the UV absorption of the target analytes and chromophore segments of macromolecules. Another advantage is that the interference by fluorescence is minimized by the excitation in the deep UV range below 250 nm. Within this presentation we report about some of our recent results highlighting the unique potential of (deep) UV Raman for biophotonic applications. We will among others show that UV-Raman spectroscopy enables a fast identification of fungal spores or clinically relevant *Candida* species. Furthermore, deep UV resonance Raman spectroscopy can be applied as an ultrasensitive analysis tool for antibiotics. Here, the bacteria-induced mushroom diseases were investigated as a general model for infection processes. The increased sensitivity due to UV excitation allows for the detection and identification of antifungal agents and toxins. In addition, UV Raman spectroscopy in combination with stable isotope labeling and 2D correlation analysis can be used for a fast pre-screening of potentially harmful bacterial strains.

Acknowledgements

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Research, Germany (BMBF), the German Science Foundation and the Carl-Zeiss Foundation are greatly acknowledged

(SPSJ-04.2) Multi-Wavelengths Ultraviolet Raman Spectroscopy for Understanding the Effect of Co-Solvents on the Structural Stability of DNA

Barbara Rossi¹, Mariagrazia Tortora¹, Andrea Mele², Jacopo Vigna³, Ines Mancini³, Alessandro Gessini¹, Claudio Masciovecchio¹; ¹*Elettra-Sincrotrone Trieste*, ²*Politecnico of Milano*, ³*University of Trento*

Although DNA is considered reasonably stable in aqueous solutions, the degradation of its structure has been observed for extended storage periods at room temperature. It has been shown that DNA is vulnerable to hydrolytic and oxidative damage in water and that non-physiological temperature, extreme pH and ionic strength or repeated freeze–thaw cycles can destroy the DNA helix structure and cause denaturation. This is a critical point especially for the molecular analysis of DNA where it is essential to optimize the storage and preservation of the sample to avoid loss of DNA quality. For this reason, the use of co-solvents such as Ionic liquids (ILs) and deep eutectic solvents (DES) for stabilizing and preserving the native structure of DNA over the long term may be envisaged for biotechnological and biomedical applications in the near future. In this contribution, we will address the question of the effectiveness in stabilizing native structure of DNA exerted by DES as novel eco-sustainable alternative to the ILs. Although these solvents are claimed to have similar physicochemical properties, recent studies evidenced that ILs and DES exhibit substantial differences in the solvation behavior of bio-macromolecules such as DNA. Multi-wavelength UV Resonance Raman spectroscopy enables the measurement of experimental quantities directly related to pair hydrogen bond strength and base stacking forces in nucleic acid strands, thus allowing to detect defined structural transitions of DNA that involve specific base-tracts. Our study reveals a more effective thermal-protective effect operated by choline-based DES on the double-helix structure of DNA respect to imidazolium-based ILs. This finding has been related to the establishment of preferential H-bonds interactions between specific DES moieties and the guanine and adenine bases in the DNA groove that lead to a more effective stacking between of these bases even at high temperature values. The results of this investigation could facilitate the designing of effective stabilizing eco-friendly organic co-solvents for their exploitation in biomedical and life science field.

(SPSJ-04.3) Continuously Tunable Wavelength, CW Deep UV Laser for Raman Spectroscopy

Ryan Roppel¹, Sergei V. Bykov¹, Sanford A. Asher¹; ¹*University of Pittsburgh*

We report on a revolutionary CW continuously tunable deep UV laser for UV resonance Raman spectroscopy pioneered by Spectra Physics and Sirah Lasertechnik. This laser enables high signal to noise deep UV Raman measurements without being plagued by high pulse energy non-linear phenomena typically associated with pulsed lasers. With a wide tunable range of 206-350 nm, a variety of chromophores can be excited to provide deep insight into electronic and vibrational molecular structure.

(SPSJ-04.4) UV Resonance Raman Studies of Tryptophan in Proteins

Judy Kim¹, Chanin Tangtartharakul; ¹*UC San Diego*

UV Resonance Raman (UVR) spectroscopy is a valuable tool for studies of biological systems. The ability of UVR to report on non-covalent interactions of residues, such as local hydrophobicity and hydrogen-bonding of tryptophan, makes this technique a powerful one for studies of complicated systems, including membrane proteins. In this presentation, we revisit the UVR spectra of tryptophan in proteins and share new insights that augment previous interpretations of the spectra. The results highlight the utility of UVR to reveal local molecular details in complex environments.

(SPSJ-04.5) Hyphenation of Raman Microspectroscopy and Field-Flow Fractionation for Analysis of Nanoplastics

Natalia P. Ivleva¹, Maximilian Huber¹, Christian Schwaferts¹, Florian Meier², Martin Elsner¹; ¹*Technical University of Munich (TUM)*, ²*Postnova Analytics GmbH*

On-line coupling of Raman microspectroscopy and field-flow fractionation is novel promising approach for nanoplastic analysis

Nanoplastics (NPLs, plastic particles <1 μm) represent an emerging topic of relevance and importance to environmental and food science as well as to human toxicology. On the one hand, these tiny particles may penetrate through cell membranes. On the other hand, due to their large surface-to-volume ratio, they may sorb large amounts of external chemicals and, therefore, are assumed to have the Trojan-horse effect in the transport of contaminants. While field-flow fractionation (FFF) can be efficiently applied for the characterization of particle sizes, the crucial particle identification as polymer cannot be achieved based on elemental composition analysis (using e.g., ICP-MS). This task can be performed by Raman microspectroscopy (RM), which provides characteristic fingerprint spectra and (due to its insensitivity to water) is applicable for wet samples.

In this presentation, the on-line coupling of RM to FFF for size-resolved chemical characterization of particles will be discussed. To implement this hyphenation, we solve the common limitation of low Raman signal from particles in diluted suspensions by employing an optical tweezer-based particle retention. The developed flow-cells were tested for suspensions of mono- and polydisperse particles with concentration of around 1 mg/L (10^9 particles/L), sizes of 100 nm – 5 μm , and different particle materials (i.e., polystyrene (PS), poly(methyl methacrylate) (PMMA), polyethylene (PE), SiO₂, TiO₂ and Fe₂O₃). Subsequently, the on-line coupling was realized for asymmetric flow FFF (AF4, suitable for size-based separation of particles in the size range of 1 nm – 1 μm) or centrifugal FFF (CF3, suitable for size- and density-based separation of particles in the size range of 10 nm – 50 μm) together with UV and multi-angle light scattering (MALS) detectors – thereby providing physical characterization via the MALS detector and chemical information via RM. To further increase the sensitivity of analysis, we currently developing different approaches for preconcentration/ enrichment of NPLs and working on the set-up optimization. On-line coupling of RM and FFF can open new possibilities for applications in fields far beyond the

NPL research, where information on chemical composition of (complex) particles, including (in)organic and (micro)biological origin, is desired together with robust data on particle size/size distribution.

Poster Presentations

Monday Poster Session - ART/ARCH

(Mon-P01) **Silcrete Geological Source Discrimination with Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry for Minimally Destructive Archaeological Stone Tool Provenience Studies**

Andrew M. Zipkin¹, Jayde N. Hirniak², John K. Murray²; ¹*Eurofins EAG Laboratories*, ²*Arizona State University*

Silcrete is a soil duricrust that has been used as toolstone since at least the Middle Stone Age and is one of the earliest mineral resources to have been heat treated to improve material properties. In the last two decades, there have been substantial advances in the detection of heat treated silcrete artifacts, as well as promising developments in geochemical provenience research. Provenience, or sourcing, studies of stone artifacts typically rely on measuring concentrations of chemical elements in geological source samples from known locations and archaeological artifacts of unknown origin. Elemental concentrations are a form of “closed” compositional data in which an increase in one element requires an equivalent decrease in the concentrations of the other elements present. This can yield unique geochemical “fingerprints” that permit multivariate statistical discrimination among sources and predictive assignment of artifacts to a source.

Here, we present pilot research demonstrating the feasibility of using a powerful and minimally invasive technique, Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS), to measure elemental concentrations in 30 silcrete samples from three geological sources in coastal South Africa and discriminate among those sources. Solution ICP-MS and ICP-Optical Emission Spectroscopy have been used successfully for silcrete provenience studies but are destructive techniques that require laborious mechanical and chemical preparation of the digestion-resistant silcrete. For this pilot study, we sub-sampled each piece of silcrete for solution ICP-MS. Concentration data for a sub-set of the measured elements were used to discriminate among the three sources. The reserved undigested portion of each sample was embedded in epoxy, polished, and ablated with five replicate scan lines. Rather than measuring fully quantitative concentrations by LA-ICP-MS, which requires knowing the concentration of a major element for use as an internal standard, we acquired semi-quantitative data externally calibrated to silicate glass reference materials. Concentrations for trace elements were then ratioed to the measured silicon concentration in each sample, effectively normalizing the data without a true internal standard. Statistical analysis of the ratio data was able to recapitulate the source discrimination achieved with the solution ICP-MS data set.

(Mon-P02) **Application of Portable LIBS and XRF to Analysis of Archaeological Artifacts**

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Laser-induced breakdown spectroscopy (LIBS) and X-ray fluorescence (XRF) provide rapid elemental composition of solid samples. These techniques have applications in archaeology for differentiation of similar, unidentified specimens. However, archaeology is often practiced without the use of analytical instrumentation. Elemental analysis can support archaeological digs by

supplementing traditional field methods with empirical information. Further, portable LIBS and XRF instruments offer the ability to perform analysis *in situ* and inform the direction of an excavation. In this poster, results from two archaeometry projects exploring the use of portable elemental-analysis instruments are presented.

In one case, handheld LIBS was used to analyze co-mingled human bones to organize skeletons. Error-prone and subjective methods are typically used to identify and separate co-mingled skeletal remains. It is hypothesized that LIBS can provide a viable confirmatory complementation of this process. The first step in determining the feasibility of this application was to compile a dataset of LIBS spectra from a set of distinct skeletons. Skeletal remains from Sai Island, Sudan were used to generate samples from pre-selected bones and pre-determined sites on each bone. Over 600 measurements were collected from ten skeletons. The dataset, consisting of spectra and skeletal site information, was analyzed using pattern recognition methods to discriminate among individual skeletons. The purpose is to show that LIBS is a viable method of assigning co-mingled skeletal remains to individuals with or without reliance on sight or fit.

In another example, handheld XRF was used to examine artefacts at the excavation site of the former Cataract House Hotel in Niagara Falls, NY. Multiple spectra were collected from two plaster walls, which are believed to have been built during separate additions to the hotel. Analysis of XRF spectra revealed unique elemental contents, providing evidence to support this hypothesis and link plaster recipes to specific time periods. Laboratory-based XRF and LIBS measurements of these samples corroborated field results. Additional samples collected from the site included flakes of green pigment, which were analyzed using several spectroscopic methods, including XRF, Raman, and infrared spectroscopy, to reveal information about the pigment's origin.

(Mon-P03) The use of time of flight ICP-MS and very fast washout laserablation systems to accurately image major and minor isotopes as well as elemental ratios in geological samples
Lukas Schlatt¹, Phil Shaw¹; ¹*Nu Instruments*

The ability for multi-elemental analysis in solid samples using rapid and direct methods is a key point in the development of analytical science. Over the years, *in situ* techniques based on Laser Ablation and Inductive Coupled Plasma Mass Spectrometry (LA-ICP-MS) have been widely used rapidly evolving into well-established, mature powerful tools for direct, high sensitivity and precision, and high lateral resolution analysis in numerous fields such as geology, biology, metallurgy, environmental sciences, etc. Significant research and advances continue to thrive to achieve the fastest, most accurate and efficient analysis. The fast acquisition speeds of the full elemental mass spectrum make a TOF-ICP-MS the most appropriate instrument for the fast examination of high-resolution elemental maps using the newest laser ablation systems. The often mentioned downside of a TOF-ICP-MS is its limited dynamic range, especially if all examined isotopes have to be examined. Using beam attenuation a novel technique was developed to create images showing elements between 100% and sub-ppm levels in one image. Examples of this technique will be shown and normalization techniques discussed. Furthermore, specific tuning can allow for the optimal detection of isotope ratios. Examples showing the differentiation of lead uranium ratios in Zicons at low micrometer resolutions will be shown. Combining the various acquisition methods in an automated fashion allows for the detailed examination of various samples in a short time with minimal input from the user.

(Mon-P04) Confocal Raman Microscopy for the Detection of Calcium Phosphates in Fluorescent Soil Matrices

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Its chemical specificity combined with high spatial resolution make confocal Raman microscopy a versatile tool for the spatially resolved analysis of a variety of specimens. The confocal geometry, ideally combined with long excitation wavelengths in the near-infrared range, is also very beneficial when investigating highly fluorescent natural samples, e.g. soil. In this study, we have applied confocal Raman microscopy with 785 nm excitation for the detection and identification of selected calcium phosphates amended to different soil matrices. The soil specimens used for our study comprise an arable soil from an Ap-horizon, loess from a C-horizon and Luvos® healing earth. Each of the soil matrices was spiked with calcium phosphate monobasic, calcium phosphate dibasic and tricalcium phosphate at a concentration of 100 μmol per gram soil, a concentration level with practical relevance for the fertilizer application zone (fertosphere) within soil. Additionally, pure calcium phosphates were analyzed to generate a set of reference spectra for their subsequent identification within the soils. Based on this approach, we could demonstrate the spatially resolved detection of each of the three phosphates in all investigated soil types. Control samples without added phosphates furthermore showed the potential of confocal Raman microscopy for the identification of intrinsic constituents within highly fluorescent soils. Detected substances include the silicates quartz and feldspar as main components within many soils but also carbonates (calcite, dolomite) and phosphates (hydroxyapatite). Based on the closely neighbored symmetric phosphate stretching Raman signals of hydroxyapatite at 962 cm^{-1} and tricalcium phosphate at 958 cm^{-1} an analytical strategy for the discrimination between these two substances was developed. The combined information of signal position and signal full-width-at-half-maximum obtained through Lorentzian curve fitting enabled a successful distinction in case of the simultaneous presence of both phosphates. These results demonstrate the potential of confocal Raman microscopy for the detection and identification of intrinsic soil minerals as well as phosphates added to various fluorescent soils. This study was funded by the Deutsche Forschungsgemeinschaft (DFG) under contract 328017493/GRK 2366 and the Federal Ministry of Education and Research (BMBF) under contracts 031A564C, 031B0513C, 01IO1623 and 16FMD02.

(Mon-P05) **In situ SEM-EDS-Raman investigation of ancient microfossils and their mineral matrix**

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Ancient fossilized microorganisms of the earliest life on Earth provide key perspective on multiple facets of Earth's development. It is believed that microorganisms that evolved in the Archean era played a key role in oxygenating the atmosphere and forming many iron-rich deposits that modern mining relies on today. Therefore, insights into these microfossils provide understanding of the evolution of early life and historical context for modern conservation of natural resources. Because microorganisms were the first type of life to evolve on Earth, they are thought to be the mostly likely type of life to find elsewhere in the universe, so understanding the early history of life on Earth can even assist in the search for extraterrestrial life.

One issue in the study of microfossils is that they frequently contain many of the same elements and structures as their mineral matrix, so can sometimes appear similar to other naturally-occurring mineral deposits. For example, micro-scale cracks that slowly fill with carbonaceous deposits can sometimes resemble microfossils. Therefore, it is helpful to have multiple types of analysis to conclusively differentiate microfossils from the surrounding mineral deposits. This work uses

scanning electron microscopy with x-ray elemental analysis (SEM-EDS) along with in situ Raman spectroscopy to study 2.5 billion-year-old microscopic structures from the Kaapvaal craton in South Africa to confidently identify these as microfossils and explore what additional insights can be gained by the joint application of SEM, EDS, and Raman spectroscopy.

Monday Poster Session - FORENS

(Mon-P06) Multivariate factor analysis to study the variations of ignitable liquid GC/MS profiles during the weathering process

Briza Marie R. Dedicatoria¹, Shruithi Perna¹, Ngee Sing Chong¹, Mengliang Zhang¹; ¹*Middle Tennessee State University*

In most arson cases, fire is initiated by employing ignitable liquids (IL). Therefore, in the arson investigation process, the analysis of ignitable liquid residues (ILR) at the crime scene plays a key role in identifying the cause of the fire and distinguishing the IL used to initiate the fire. Weathering of IL takes place at any temperature; as a result, the relative components in the IL mixture will be altered, which causes it difficult for the arson investigators to compare the weathered to unweathered IL. In the weathering process, two factors, including weathering degree and temperature, were found to affect the chemical profiles of ILR significantly. This study aims to investigate the impacts of weathering degree and temperature factors on the GC/MS total ion chromatogram (TIC) profiles by chemometrics.

Analysis of variance-principal component analysis (ANOVA-PCA), firstly introduced by Harrington et al. to discover biomarkers in the proteomic study, has been used in this study for multivariate factor analysis. This method separates the variation of the experimental hypothesis from other potentially confounding sources of variation, and it is ideal for investigating the problems with multi-experimental factors such as the weathering degree and temperature effects in the ILR weathering process. IL, such as paint thinner, was weathered at three different temperatures, 30 °C, 90 °C, and 210 °C, to different degrees of weathering at 30-99% mass reduction of IL before being analyzed by the GC/MS. The data indicate that the weathering percentage is the major factor that is responsible for the variance of the TIC data. It was found that the retention time alignment to TIC was critical to studying the weathering effects of IL, and with ANOVA-PCA, the impact of weathering degree and temperature factors on the TIC profiles were statistically quantified. Our study has demonstrated an innovative approach to better understanding the compound profile changes of IL during the weathering process.

(Mon-P07) Formation of spermine phosphate hexahydrate crystals in semen probed by Raman microspectroscopy

Sonivette Colón-Rodríguez¹, Igor K. Lednev¹; ¹*University at Albany, State University of New York*
Semen evidence can be of critical importance in sexual assault cases. Semen confirmatory tests rely on microscopically confirming sperm cells or detecting seminal fluid proteins via immunoassay tests. Serological methods that utilize antibodies can also be used in cases of azoospermia or oligospermia for seminal plasma detection. However, these tests are destructive and can result in false results. Our laboratory has developed a universal approach for the identification of all main body fluids, including semen, based on Raman spectroscopy. This is a non-destructive test, and the traces of semen could be further used for DNA profiling. The presence and identification of Spermine Phosphate Hexahydrate (SPH) crystals in semen samples by Raman spectroscopy were first published by our group in 2019. Here we present a more extensive study of the formation of SPH crystal in fresh human semen samples due to freezing at -10 °F (-23 °C). It was found that freezing and thawing stimulate SPH crystallization, whereas no SPH crystals were detected in the control sample at room temperature. The

cycles of freezing/thawing showed SPH crystal formation after 7 hours when the sample was left alone during the freezing time, but only 4 hours when the sample was thawed every 3 hours. It was concluded that the formation of SPH crystals in fresh samples collected and stored for confirmatory analysis could be a convenient target analyte for semen identification by Raman microspectroscopy.

(Mon-P08) A Universal Test for the Forensic Identification of All Main Body Fluids Including Urine

Bhavik Vyas¹, Lenka halamkova¹, Igor K. Lednev¹; ¹*University at Albany, State University of New York*

A critical aspect of forensic investigation is to detect and identify body fluid stains and preserve them for DNA extraction. The body fluid stains commonly found at a crime scene include blood, saliva, semen, sweat, vaginal fluid, and urine. Identification of a stain can be difficult as current methods are body fluid specific and mostly destructive. In an effort to develop a universal, confirmatory and nondestructive approach that can identify and differentiate body fluids Muro et al. (2016), combined Raman spectroscopy and chemometrics to build a statistical model which could differentiate peripheral blood, saliva, semen, sweat, and vaginal fluid. However, this model did not include urine. Urine can be vital evidence in cases of correctional officers being assaulted with urine bombs by prisoners, as well as in sexual assault cases. Recent studies have also shown that DNA can be extracted from a dried urine sample. In this study, we have combined the Raman spectral dataset collected by Muro et al. with Raman spectral data collected from 27 urine samples from different donors to build calibration and validation datasets. Chemometrics was applied to build an enhanced statistical model which can identify and differentiate all main body fluids including peripheral blood, saliva, semen, sweat, vaginal fluid, and urine. This classification model offers a universal, single-step, non-destructive, and robust technique with 100% accuracy for sample identification.

(Mon-P09) Identification and Discrimination of Fibers by Raman Spectroscopy

Sergey Mamedov¹; ¹*HORIBA Scientific*

Raman microspectroscopy is very applicable in the field of forensics. It uses a technique that offers a non-destructive and non-contact method of analysis. Only a small amount of sample is required, and little or no sample preparation is necessary. It allows for trace analysis, whereas sampling can be done directly through transparent evidence bags and packaging, such as glass and plastics. It covers a wide spectral range from 10 cm⁻¹ to 4000 cm⁻¹, making the technique ideal for identifying organic and inorganic substances, including fibers, drugs, pharmaceuticals, explosives, inks, paint, etc. Raman microspectroscopy also allows identification of the components of inhomogeneous samples and obtaining automated high-definition Raman mapped images.

To aid law enforcement personnel and the public at large, investigations have been geared toward the ability of Raman microspectroscopy to identify a variety of polymers used in fibers. This is very important, as the presence of fibers at a crime scene has often been instrumental in solving crime. “Fingerprints” of nylon 6, Kevlar, polystyrene, PET, poly-propylene, and some other fibers along with different types of nylon (nylon 6, nylon 6/6, nylon 12, and others) will be highlighted in this paper, as well as the ability to identify fiber mounted on a substrate. The capability of Raman spectroscopy to differentiate between fibers of similar chemical structures will be demonstrated.

Spectral data of the fibers were collected using 532 nm, 633 nm, and 785 nm laser excitations. A comparison of the Raman spectra of the fibers taken with different excitation wavelengths will be discussed.

(Mon-P10) Stand-off Raman Spectroscopy: A Novel Method for the Detection and Identification of Body Fluid Traces

Lamyaa M. Almeahadi¹, Igor K. Lednev¹; ¹*University at Albany, State University of New York*

Biological stains play a significant role in crime scene investigations as a major source of DNA evidence. Most in-field tests designed for body fluids identification are non-universal and presumptive, with possible false positives. Thus, there is a need for modern technology to improve the body fluids identification process. Raman spectroscopy has a great potential for becoming a universal tool for confirmatory identification of body fluid traces. We introduce stand-off Raman spectroscopy combined with chemometrics as a new method for detecting and identifying body fluids. Our work demonstrates the applicability of stand-off Raman spectroscopy to detect and identify body fluids using a hand-held Raman spectrometer allowing in-field and remote sensing.

(Mon-P11) Universal Method for Body Fluid Identification for Forensic Purposes: The Commercialization Effort

Alexis R. Weber¹, Igor K. Lednev¹; ¹*University at Albany, State University of New York*

The ability to identify body fluid traces at crime scenes, while preserving any DNA present, is critically important in forensic science. Currently in forensic science laboratories, the identification can be difficult and many of the current techniques are specific to one body fluid. Additionally, typical biochemical methods are destructive – preventing any further analysis. When there is a problem within the scientific field, research laboratories are the main group to solve this problem. After conducting research in the laboratory, the next step in the process is to commercialize the research. Commercialization is bringing a product to market and selling it for financial gain. Within the Lednev Laboratory, in order to develop a universal, confirmatory, nondestructive, approach that can be used to differentiate and identify body fluids, the specificity of Raman spectroscopy was combined with the analytical power of statistical modeling.

All six forensically relevant body fluids (blood, semen, saliva, sweat, urine, and vaginal fluid) were successfully discriminated by coupling Raman spectroscopy and chemometrics. This technique is both reliable and nondestructive, offering substantial advantages over the current techniques used to identify body fluids. This development of this product has occurred over several years to prepare it for sale, with the culmination of this being the creation of the start-up company SupreMETric LLC. SupreMETric's mission is to streamline the forensic analysis of biological stains by creating a universal nondestructive method for the identification of all main body fluids. This presentation covers the process from research to commercialization process of this technology.

(Mon-P12) Surface-Enhanced Raman Spectroscopy Enables Highly Accurate Identification of Different Brands, Types and Colors of Hair Dyes

Samantha Higgins¹, Dmitry Kurouski¹; ¹*Texas A&M University*

Hair is present at nearly all crime scenes. Forensic analysis of hair can be used to establish a connection between a suspect and a crime scene or demonstrate the absence of such connection. Almost half of people around the world color their hair. However, there is no robust and reliable forensic approach that can be used for a confirmatory analysis of artificial colorants present on hair. A growing body of evidence suggests that surface-enhanced Raman spectroscopy (SERS), a modern analytical technique, can be used to detect and identify colorants present on hair. In the current work, we examined the

potential of SERS in identification of more than 30 different colorants. We found that the accuracy of detection and identification of individual hair colorants is 98%, on average. We also investigated the extent to which SERS can be used to differentiate between different brands and types of colorants, as well as to identify hair color regardless of the type and brand of the colorant used to dye hair. Our results showed that individual colorants could be identified with on average 98% accuracy, whereas different brands can be predicted with nearly 100% accuracy. We also found that SERS offered nearly 100% accurate identification of the type of the colorant and on average 97.6% accurate prediction of the hair color. These results demonstrate that SERS can facilitate the forensic analysis of hair providing highly important information about the artificial colorants present on the analyzed specimens.

(Mon-P48) Probing Menstrual Bloodstain Aging with Fluorescence Spectroscopy

Anna Wójtowicz¹, Alexis R. Weber², Renata Wietecha-Posłuszny¹, Igor K. Lednev²; ¹*Jagiellonian University*, ²*University at Albany, State University of New York*

Menstrual blood (MB) is a common and important type of forensic evidence, especially in sexual assault cases. MB consists mainly of peripheral blood, vaginal fluid, and endometrial cells of the uterine wall. In forensic investigations, the differentiation of menstrual and peripheral bloodstains is crucial, as it indicates whether the blood present is the result of a tissue damage from an assault or a natural cause, such as menstruation. The next important step is to establish the time since deposition (TSD) of a bloodstain, which can be the key to determining time when the crime was committed, identifying the biological stains related to the crime, and isolating those involved.

To develop a robust forensic method for determining the TSD of a bloodstain, it is necessary to understand the underlying biochemical mechanisms involved in its aging. Fluorescence spectroscopy, a promising spectroscopic method for bloodstain analysis, was used to probe the biochemical changes that occur over time in menstrual bloodstains [1]. During the study, menstrual blood samples were deposited on aluminum-covered glass slides and aged under ambient conditions. Fluorescence spectroscopy data was collected within 24 hours after the deposition. The steady-state fluorescence spectra were found to change significantly during this TSD. The mechanism underlying the change in fluorescence has been proposed to involve the kinetic transformation of three fluorophores: tryptophan, nicotinamide adenine dinucleotide, and flavins. Moving forward, by measuring changes in these compounds as a factor of time, it will be possible to approximate the TSD of menstrual bloodstains. The observed spectral changes, their kinetics, and variability between donors will be discussed.

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[1] A. Wójtowicz, A. Weber, R. Wietecha-Posłuszny, I.K. Lednev, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 248 (2021), 119172.

Monday Poster Session - IR

(Mon-P13) A Deep Convolutional Neural Network for bond/functional group identification from gaseous Infrared Spectra

Fernando Fischer¹; ¹*Universidad Católica Boliviana San Pablo*

One dimensional Convolutional Neural Networks are powerful at processing raw spectra due to their reduced vulnerability from shifting input values. In this paper a Neural Network of such type is trained to identify 9 Bonds/Functional groups from a given IR Spectrum. 4836 Spectrums from the NIST Database were used for training and evaluation of the model. A tutorial for the implementation of the model can be found at <https://github.com/fernandofischer98/pytorch-spectrum-analysis>.

(Mon-P14) Quantum Cascade Laser-based Rapid Vibrational Circular Dichroism Spectroscopy

Yamuna Phal¹, Rohit Bhargava¹, Ruo-Jing Ho¹; ¹*University of Illinois Urbana-Champaign*

Vibrational Circular Dichroism (VCD) spectroscopy is an emerging technique that can enable enantiomeric separation by targeting the underlying chiroptical phenomena, which arises from preferential absorption of one direction of circularly polarized light versus another. The major challenges in practical implementation of a rapid VCD system arose from the limited signal-noise ratio (SNR) and the long acquisition times of Fourier transform Infrared (FT-IR) VCD spectrometers. Using the high power spectral density, SNR and spectral tunability of quantum cascade laser (QCL) sources, we show that a rapid measurement modality, capable of assessing VCD along with IR absorbance measurements are possible. Our work provides a platform for future studies of monitoring minute changes in chiroptical activity that have major implications for enantiomeric studies. Such systems have the potential to further improvise drug delivery control systems with diagnostic capability beyond that possible today and aid the development of molecular therapeutics.

(Mon-P15) A Deep Learning Encoder-Decoder model for SMILES sequence generation from gaseous Infrared Spectra

Fernando Fischer¹; ¹*Universidad Católica Boliviana San Pablo*

A deep learning model for generating molecular structure from Infrared Spectra is presented. The model follows the well-known translation Encoder-Decoder Structure. The model was trained using a Deep 1D Convolutional Neural Network as the Encoder and a Recurrent GRU Neural Network as the Decoder. The Model generates a SMILES sequence from a given Spectrum. A tutorial for the implementation of the model can be found at <https://github.com/fernandofischer98/pytorch-spectrum-analysis>.

(Mon-P16) Rapid Screening of Clover Honey Adulteration with Infrared Spectroscopy and Chemometrics

WILLIAM LIMM¹, Sanjeeva R. Karunathilaka¹, MAGDI MOSSOBA¹; ¹*FDA*

Due to its high price, increased consumption, and limited production, honey has been a main target for economically motivated adulteration (EMA). An approach combining Fourier- Transform infrared spectroscopy (FTIR) and chemometrics was evaluated to develop a rapid screening tool to detect potential EMA of honey with either rice or corn syrup. A single class soft independent modeling of class analogy (SIMCA) model was developed using a diverse set of commercial honey products and an authentic set of honey samples collected at four different USDA honey sample collection locations. The SIMCA model was externally validated with a set of calibration-independent authentic honey, typical commercial honey control samples, and those spiked with rice and corn syrups in the 1-16% concentration range. The authentic honey and typical commercial honey test samples were correctly predicted with a 91% classification rate. High accuracy was found

in predicting the rice and corn syrup spiked samples above 7% concentration range, yielding 97.7% and 94.8% correct classification rates, respectively. This study demonstrated the potential for a rapid and accurate infrared and chemometric method that can be used to rapidly screen for either rice or corn adulterants in honey in less than 5 minutes.

(Mon-P17) Measurement of Workplace Aerosols by simultaneous IR and Raman using Optical Photothermal Infrared Spectroscopy

Vasileia Vogiaz¹, Nicholas E. Pugh², Orthodoxia Zervaki¹, Pramod Kulkarni¹; ¹*NIOSH / CDC*,
²*South Dakota School of Mines*

Long term inhalation exposure to aerosols such as respirable crystalline silica (RCS) or asbestos particles in workplace atmospheres can pose health risks. Fast and accurate identification and quantification of these chemical agents at low detection limits is important to monitor exposure levels of workers. In this study, we probed feasibility of the Optical Photothermal Infrared Spectrometer (O-PTIR) to quantify the air concentration of these aerosols. O-PTIR can provide hyperspectral images with simultaneous IR and Raman spectra at the same time and from the same spot on the particulate sample. The paired vibrational spectra can improve specificity of aerosol detection, especially in complex workplace samples with multicomponent analytes and mineral interferences. The study involved development of an analytical methodology for RCS detection and quantification at low concentrations, as well as estimating detection limits and measurement uncertainty. To optimize sample preparation, we tested three types of filter substrates and three different air sampling techniques. These included total filter collection and two spot sample collection techniques. Preliminary results include successful acquisition of both IR and Raman spectra for RCS, and generated calibration curves for all three collection techniques. The O-PTIR based method can provide direct-on-filter quantification of RCS at detection limits below current exposure limits. Measurement of different types of asbestos fibers showed that the technique can provide selectivity in fiber counting with respect to fiber shape and aspect ratio. The O-PTIR method can also provide chemical specificity for asbestos mineral using Raman and/or IR signature.

(Mon-P18) The Effect of Particle Size on Measurement Uncertainty of Analyte Quantification in Infrared Spectroscopy

Kabir Rishi¹, Bon-Ki Ku¹, Chen Wang¹, Vasileia Vogiaz¹, Orthodoxia Zervaki¹, Pramod Kulkarni¹;
¹*NIOSH / CDC*

Infrared (IR) absorption measurement is commonly used for quantification of chemical components in particulate samples. Quantification of analyte content requires calibration using a standard reference material. For particulate samples, because light scattering and absorption are particle size dependent, the calibration curve generated is specific to the reference material used. Measurement uncertainty can be significant if the unknown aerosol has a different size distribution compared to that of the calibration aerosol. Previous studies have probed the effect of particle size on analyte quantification in IR absorption measurements, however, they lacked adequate size-resolved measurements over an extended particle size range to provide meaningful assessment of size effects and uncertainties.

The objective of this study was to probe the measurement artefacts related IR absorption measurement of particulate samples collected on filters with respect to their particle size and their spatial distribution density on the filter. Aerosolized a-quartz standard reference material from NIST was size classified using an 8-stage Andersen cascade impactor in the respirable size range to obtain test samples with

narrower size distribution but distinct mean particle diameters in the range 0.4 – 4 μm . Each size selected fraction was extracted into an aqueous suspension and was redeposited on a PVC filter for absorption measurement using transmission mode IR. NIST traceable polystyrene spheres with different sizes were also probed. The peak IR absorption measurements, normalized by the surface mass density of analyte, was used to compare absorption characteristics of samples with different particle size distribution. These measurements compared well with the theoretical predictions from single particle Lorentz-Mie light scattering theory. The complex refractive index was observed to strongly influence the size-dependent absorption trend in the IR wavelength range studied. Comparison of experimental and theoretical absorption spectra and implications for method calibration and overall measurement uncertainty will be presented and discussed.

(Mon-P19) Examining The Impact Of Gold Nanoparticles On Amylin Aggregation Via Two-dimensional Infrared Spectroscopy

Kayla Hess¹, Sophia Vogelsang¹, Nathan Spear¹, Janet Macdonald¹, Lauren E. Buchanan¹;
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Nanoparticles (NPs) have grown highly prevalent in multiple industries, increasing concerns about potential health and safety risks. As NPs are being considered for therapeutic purposes, it is critical to characterize how they interact within the body. Upon introducing NPs to a biological media, proteins adsorb onto NP surfaces causing the formation of a protein corona. The interactions between proteins and the NP surface can induce structural changes and subsequently alter native protein function. Thus, understanding how NPs interact with different proteins is necessary for isolating potential health hazard risks. Amyloid proteins are characterized by the formation of extended β -sheet structures known as fibrils and are associated with diseases such as Alzheimer's and type II diabetes. Over the course of aggregation, cytotoxic intermediates known as soluble oligomers form; therefore, concerns exist that introducing NPs could prolong the lifetime and cytotoxicity of such species. Currently, the mechanism by which NPs modulate amyloid aggregation is unknown. To determine the mechanism, we have utilized a combination of two-dimensional infrared spectroscopy and isotope labeling to resolve how gold NPs alter the secondary structure and aggregation kinetics of human islet amyloid polypeptide (hIAPP). The use of isotope labeling has allowed for residue-specific interactions to be monitored with and without NPs present over the course of aggregation. Isotope labels placed in different locations throughout hIAPP in individual peptides have revealed specific oligomeric species during aggregation and structural polymorphs in the final fibril form. Our initial results suggest that gold NPs inhibit hIAPP aggregation and alter the transition dipole strength of the aggregated fibrils, indicating a change in coupling within the fibrils. Kinetics studies utilizing isotope-labeled hIAPP are currently underway to fully understand how NPs alter the mechanism of aggregation and to identify the formation of oligomers in the presence of NPs.

(Mon-P20) Long short-term memory and Transformer in Classification and Correction of ATR distorted spectrum

Rui Cheng¹, Johannes Kiefer¹; ¹*Universität Bremen*

A complex mixture usually produces a distorted ATR spectrum. Especially when one or several components of the mixture have a refractive index higher than the critical refractive index of the ATR internal refraction element, the spectrum will be distorted and it is difficult to identify the correct peak positions. Therefore, a fast and accurate method for distinguishing and correcting distorted ATR spectra is highly desirable. Here, for the first time, we propose a method using long-

short term memory (LSTM) and Transformer to divide and correct the distorted ATR spectrum. These two correction methods are based on deep learning and do not require complex expert knowledge in order to provide very accurate classification and correction results for further basic research or engineering applications.

(Mon-P21) Rapid Detection of COVID-19 Using Ultra-Compact MEMS Based Spectrometer and Supervised Machine Learning

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COVID-19 is the pandemic of the decade with over 13 million deaths in 2020 and 2021. Rapid detection of the virus infection has been studied using numerous tools worldwide. In this work, infrared spectroscopy is tested as a tool for kit-free detection of the virus infection using fast and portable spectroscopic MEMS scanners, where the virus fingerprint is investigated in mid infrared region using nasopharyngeal and oropharyngeal swab viral transport medium. The ATR spectrum was collected by a drop deposition on a diamond crystal. The interpretation and statistical analysis of the data is done using iPLS discriminant analysis which is a supervised machine learning algorithm for interval selection and statistical modeling. The results demonstrate a specificity of 97 % and an accuracy of 90 %.

(Mon-P22) Using TG-IR Hyphenation for Advanced Material Insight

Samantha L. Nania¹; ¹*PerkinElmer*

Hyphenation combines two different instruments to gain analytical insights which cannot be obtained with either instrument in isolation. One of the most common hyphenation techniques is Thermal Gravimetric Analysis (TGA) in combination with an FT-IR, commonly called “TG-IR”. These two technologies have long been used to characterize materials and in some cases, the degradation products from decomposition reactions. In concert, these two techniques provide a more holistic perspective of material composition: gravimetric data provides quantitative insight, while the FT-IR acts as a chemical detector, measuring materials evolved from the sample. Users are challenged to extract the most meaningful information from the experiment, and multiple approaches are commonly employed including multivariate analysis. Example data will be presented to illuminate insights and potential workflows to navigate through outputs from such data-rich experiments.

(Mon-P23) Identification and Quantification of High-Consequence Chemical and Biological Toxin Surrogates with Infrared Spectroscopy

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Recently there has been a growing amount of emerging and existing high-consequence toxins impacting industrial, agricultural, and public safety sectors. These toxins, which include avian influenza virus, anthrax, and botulinum toxin, are high-impact biological and chemical threat agents to organisms that encounter them. With the increased occurrence of these toxins, there is a need for

rapid, fieldable methods to detect and identify such species. Infrared (IR) reflectance spectroscopy is a robust analytical technique that produces highly specific vibrational signals for chemical and biological analytes and can be used to interrogate surfaces *in-situ* without sample preparation, transportation, or storage. Reflectance IR spectroscopy provides advantages over conventional IR methods, which require the isolation and placement of the analyte in the beam path of the instrument on an IR-transparent surface.

Here, we explore the ability of attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) to quantify and distinguish surrogates of chemical and biological toxins. Chemical toxins analyzed include nodularin, microcystin, deoxynivalenol, ochratoxin A, and zearalanone. Biological agents analyzed include lipopolysaccharides from *Salmonella enterica*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and inactivated strains of herpes simplex virus 1, herpes simplex virus 2, adenovirus 2, and influenza A (H1N1) virus. Each sample was dissolved in water or ethanol, deposited onto the ATR crystal or portable substrate, and evaporated to form a film. Spectra were then collected over the 700 to 4000 cm^{-1} range with a resolution of 4 cm^{-1} . A library of spectra for each analyte was generated under a variety of conditions (e.g., surface-area coverage, concomitant species, etc.). Instrument response was linear for surface densities ranging from 20 to 160 ng/mm^2 , enabling quantification of the amount of material deposited on a surface. Multivariate statistical methods including principal component analysis (PCA) will be employed to determine the spectral regions most diagnostic for identification of each type of toxin. This work will inform the development of a portable IR reflectance instrument based on a tunable quantum cascade laser (QCL). Ultimately, this instrument should offer a way to rapidly identify toxic substances on surfaces in industrial, academic, military, or agricultural settings.

(Mon-P24) **Metabolic Fingerprinting For Diagnosis of Fibromyalgia and Other Rheumatology Disorders**

Haona Bao¹, Luis E. E. Rodriguez-Saona¹; ¹The Ohio State University

Although Fibromyalgia syndrome (FM) diagnosis has evolved over the years, changing from dependence on the presence of tender points to one that is inclusive of comorbid features, there is no uniformity of acceptance of FM amongst physicians. Many physicians lack the necessary training to diagnose this condition accurately. As a result, patients with poorly explained symptoms are often lumped into the FM category inappropriately. The Discovery of a biomarker for FM would be a critical step toward developing novel therapies. A biomarker could help in the longitudinal characterization and prognosis of FM patients. Our aim was to determine the clinical reliability of blood-based biomarkers to differentiate subjects with FM (n=90) from individuals with other rheumatology disorders including osteoarthritis (OA, n=30), rheumatoid arthritis (RA, n=30), and systemic lupus erythematosus (SLE, n=30) using IR spectroscopy techniques. Blood samples from subjects were collected by using bloodspot cards and Neoteryx tubes. Blood samples were placed onto a highly reflective slide, and spectra were collected by a portable FTIR equipped with an attenuated total reflectance (ATR) accessory and using an imaging FTIR system. Spectra were analyzed using pattern recognition to differentiate among groups and a regression algorithm for grading disease severity. The unique FTIR spectra allowed to identify FM patients from other syndromes. The discriminating power plots identify distinctive differences in spectra responsible for the separation of classes. The results demonstrate FTIR may provide particular spectral patterns associated with FM. The novel aspect of this research is to show the potential of FTIR as a rapid detection method of FM syndrome for routine clinical use with advantages including rapid, low cost, requiring small sample sizes, and easy to operate.

(Mon-P26) Visible-Near-Infrared Spectroscopy and Machine Learning Methods for the Identification of Amaranthus Species

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The possibility of rapid and non-destructive discrimination of six different *Amaranthus* species was investigated using visible-near-infrared (Vis-NIR) spectra coupled with chemometric approaches. The focus of this research would be to use a handheld spectrometer in the field to classify six *Amaranthus* sp. in various geographical regions of South Korea. Spectra were obtained from the adaxial side of the leaves at 1.5 nm intervals in the Vis-NIR spectral range between 400 and 1075 nm. The obtained spectra were assessed with four different preprocessing methods in order to detect the optimum preprocessing method with high classification accuracy. Preprocessed spectra of six *Amaranthus* sp. were used as input for the machine learning-based chemometric analysis. All the classification results were validated using cross-validation to produce robust estimates of classification accuracies. The different combinations of preprocessing and modeling were shown to have a higher classification accuracy of between 71% and 99.7% after the cross-validation. The combination of Savitzky-Golay preprocessing and Support vector machine showed a maximum average classification accuracy of 99.7% for the discrimination of *Amaranthus* sp. Considering the high number of spectra involved in this study, the growth stage of the plants, varying measurement locations, and the scanning position of leaves on the plant are all important. We conclude that Vis-NIR spectroscopy, in combination with appropriate preprocessing and machine learning methods, may be used in the field to effectively classify *Amaranthus* sp. for the effective management of the weedy species and/or for monitoring their food applications.

Monday Poster Session - MASS

(Mon-P27) Accurate identification, examination and differentiation of multielement nanoparticles using time of flight ICP-MS and sub-millisecond spectral acquisition times

Lukas Schlatt¹, Phil Shaw¹; ¹Nu Instruments

The use of nanoparticulate materials has been increasing with large amounts being released into the environment each year. Therefore, sensitive methods are needed to detect these particles. While the size detection limit has been decreasing over the years with more sensitive detection methods becoming available, completely new instrumentation to scanning mass spectrometers is necessary to examine the composition of individual particles. TOF-ICP-MS is capable of measuring the full mass spectrum continuously at acquisition speeds down to 80 μ s. Together with powerful peak integration and identification methodologies, the data from individual nanoparticles can be identified and reduced efficiently and easily. In addition to the capability to record multiple full mass spectra for every particle, examinations of nanoparticles with a reduced mass range can be recorded to gain a higher sensitivity for a smaller number of observed isotopes. This allows for the reduction of size detection limits and more detailed examination of elemental ratios in single nanoparticles. Data is shown, which differentiates a mix of various particles into clusters. This way a mixture containing various similar as well as different particles can be differentiated quickly and easily. Furthermore, the advantage of reduced mass range examinations is shown and discussed.

(Mon-P28) Optimization of Surface Assisted Laser Desorption Ionization by Studying Material Properties of Polymer Nanofibers with a Photothermal Heterodyne Imaging Setup

Ye Chan Moon¹, Zac D. Schultz², Susan V. Olesik²; ¹*The Ohio State University*, ²*The Ohio State University*

Matrix assisted laser desorption ionization coupled with time-of-flight mass spectrometry (MALDI-TOF-MS) is utilized in microbiology, forensics, and medicine as a means for identification and diagnosis due to spectral simplicity, high sensitivity, and low sample consumption. A major problem with MALDI occurs when trying to quantify small molecules (< 1000 Da) because of the interference of the organic matrix materials. Surface assisted laser desorption ionization (SALDI) overcomes this problem by using a surface instead of a matrix although the mechanisms are not extensively established in literature. The goal of this research is to understand the material properties that will optimize the SALDI performance of electrospun nanocomposite fibers. Heating is thought to be a candidate for one of the mechanisms of SALDI due to using nanocomposites with absorption peaks closer to the MALDI laser resulted in higher analyte intensities. Nanocomposites containing plasmonic nanoparticles can transfer “hot” electrons as observed in Raman characterization of electrospun polyacrylonitrile (PAN) nanofibers with gold nanoparticles. This research will use a homemade Raman spectrometer incorporated photothermal heterodyne imaging (PHI) setup to study the heating and ionization mechanisms of the polymer nanofiber SALDI mat. The properties observed will be correlated with the SALDI performance of the nanofiber substrates.

(Mon-P29) Diagnosis of Agglomeration of Crystallinity of Active Pharmaceutical Ingredients in Quartin Pills by Electro Spray Laser Desorption Ionization Mass Spectrometry Ionization
Margaret A. Sperry¹; ¹*Marian University*

In this study, quartin pills were analyzed via Electro spray Laser Desorption Ionization Mass Spectrometry Imaging (ELDI-MSI). Standardized pharmaceutical preparations with known amounts of crystallinity were imaged via ELDI-MSI to determine localities of potential crystalline morphologies. Multiple layers of pills were sampled to develop 3-D maps and determine crystallite size and location to compare to x-ray methods. ELDI-MSI has been shown to effectively analyze tablets for agglomeration, potentially due to crystalline morphology of the active ingredient. The combination of ELDI-MSI and x-ray imaging allows for a novel total analysis of tablet formulations for locations of crystals via x-ray and spatially resolved chemical identification via ELDI-MSI. Since x-ray imaging cannot confirm chemical identities of high density material, ELDI-MSI is an ideal complementary technique to provide the identity of crystalline material. ELDI-MSI is a non-selective technique, which allows for spatial distributions of all observable compounds to be concurrently imaged. Here we show the same quartin pills imaged via both x-ray and ELDI-MSI and compare results of each method to determine compatibility of results.

(Mon-P30) Diagnosis of Agglomeration and Crystallinity of Active Pharmaceutical Ingredients in Pharmaceutical Preparations of Clotrimazole by Electro spray Laser Desorption Ionization Mass Spectrometry Imaging

Kelsey K. Ramp¹, Patrick A. McVey¹; ¹*Marian University*

Clotrimazole is a poorly soluble, BCS Class II drug, whose bioavailability can be lowered when found in the crystalline form in tablet formulations. In previous studies, Electro spray Laser Desorption Ionization Mass Spectrometry Imaging (ELDI-MSI) has been shown to be capable of detecting crystalline active pharmaceutical ingredients (APIs) as areas of agglomeration, with no sample matrix application. Areas of crystallinity are identified by co-localizing areas of high-intensity API signal with signal expected from typical amorphous API and surfactant distribution.

Amorphous API in the tablet will co-localize with signal from surfactants, because during tablet formulation surfactants are used to aid in the processing of the final tablet dosage form by associating with amorphous API in the tablet. Crystalline API can be identified as areas of agglomeration in the tablet which do not co-localize with signal from surfactants. In order to confirm the presence and location of crystalline API, all data must be compared to x-ray images. However, due to the inability of x-ray to determine the chemical identity of a high density signal, ELDI-MSI is an essential method for confirming crystalline API in a tablet formulation. This study focuses on diagnosing agglomeration and crystallinity in standard pharmaceutical preparations of clotrimazole across multiple layers, and broadening the use of ELDI-MSI in diagnosis of agglomeration and crystallinity in tablet formulations. ELDI-MSI is an important diagnostic tool for the pharmaceutical industry and consumers, as crystallinity and decreased bioavailability of poorly soluble drugs in tablet formulations can lead to health complications. This can be particularly problematic for consumers taking a low dose of an in-soluble drug needed for the prolonged treatment of a chronic health condition.

(Mon-P31) The Development of High Throughput Metabolomics To Aid The Synthetic Biology Design-Build-Test-Learn Cycle

Georgie Barrett¹, Susan Rosser¹, Karl E V Burgess¹; ¹*University of Edinburgh*

The basis of synthetic biology is the design and construction of new biological systems and the re-design of existing natural systems for specific purposes, for example engineering a strain to increase the yield of a high value product. Due to the complex nature of biological systems, synthetic biology often requires several iterations and cycles of the design-build-test-learn cycle to see improvements in a bioprocess. The current major bottleneck in the cycle is the “test” phase. This is due to the large number of conditions and strains that need to be analysed to determine optimal growth and yield, leading to time-consuming testing processes. To overcome this bottleneck we have explored increased throughput via low cost LEGO automation, flow injection mass spectrometry and the use of specific inhibitors to probe the metabolism of a strain of *Escherichia coli* NST74, a feedback deregulated phenylalanine overproducer. Our LEGO automation system can create cell culture assays, inhibitor assays and perform metabolomic extractions on 96 well plates. This reduces the time taken to culture the bacteria, perform metabolite extractions and generate samples. Samples were analysed via flow injection mass spectrometry using targeted metabolomics, and more specifically multiple reaction monitoring (MRM) on a triple quadrupole instrument. Each run takes less than 3 minutes per sample allowing a full 96 well plate to be analysed in less than 5 hours. This enables rapid analysis of the effects of the cell culture assays or inhibitor assays, as well as enabling quantification of the yield of the product – in this case phenylalanine. Using an example of tryptophan as an inhibitor of anthranilate synthase, it was hypothesised that this would drive flux towards increased yield of phenylalanine. The results show that 42 metabolites in the glycolysis, pentose phosphate and shikimate pathways were detected, and a 3.1 fold increase of phenylalanine was found with 1mM tryptophan. These results show that the bottlenecks of the design-build-test-learn cycle can be ameliorated, and that inhibitor assays are useful to pinpoint critical pathways and discover other targets for synthetic biology.

(Mon-P32) Quantitation of Boron in Carbon Rich Matrices via Alkoxylation Gas Chromatography Mass Spectrometry as an Alternative to Plasma Spectrochemical Analysis

Matthew Masters¹, Ron Tecklenburg¹, Eb Debrah¹; ¹*The Dow Chemical Company*

Plasma source spectrometry with mass detection is one of the key analytical tools for the analysis of trace elements. It has many useful attributes such as low detection limits for most elements and wide elemental coverage among others. Like other analytical techniques interferences in ICP-MS are reasonably understood but it can often be time consuming and difficult to compensate for them especially in complex matrices. Determination of low levels of boron, a key element in semiconductor materials and in other applications is critical. Traditional sample pretreatment approaches using digestion with mineral acids can lead to analyte loss, memory effects and the increased risk of contamination at the ultra-trace concentration level. In addition, determination of boron by ICP-MS in complex matrices with high carbon content is exacerbated by the presence of the extremely high ^{12}C peak which readily obscures the adjacent ^{11}B peak. Single quadrupole ICP-MS is unable to resolve the highly elevated background signal making accurate quantitation extremely difficult. Here, an alternative approach for the quantitation of boron (as boric acid) is reported. A gas chromatographic method was used to separate the boron from the concomitant matrix through the selective alkoxylation of the boron (as boric acid) to form an alkoxy borate that is then readily quantitated using GC-MS. Using a model compound in a complex polymer matrix, the recovery for boron measured using this new method was better than 97% with a detection limit in the low ppb range. In addition to the simple sample pretreatment approach the versatility of the method lies in flexibility of the alcohol choice used in the alkoxylation reaction which allows the GC-MS analysis to be tailored depending on the matrix composition.

(Mon-P33) Characterization and Quantification of Natural and Anthropogenic Titanium Nanoparticles using single-particle Inductively Coupled Plasma Time-of-Flight Mass Spectrometry

Hark B. Karkee¹, Sarah E. Szakas², Alexander Gundlach-Graham²; ¹Iowa State university, ²Iowa State University

Titanium (Ti) nanoparticles (NPs) present in environmental samples may be naturally occurring (Ti-NNPs) or engineered (Ti-ENPs). For example, titanium dioxide (TiO_2) ENPs are common additives in products such as paints, plastics, food products, and cosmetics. These Ti-ENPs ultimately make their way to environmental compartments such as water, soil, and air. Studies have shown that TiO_2 NPs may induce cytotoxic effects with a slight inhibition of cell growth at higher concentrations.¹ Therefore, methods to quantify Ti-ENPs in the environment are of interest.

In recent years, single-particle inductively coupled time-of-flight mass spectrometry (spICP-TOFMS) has been used to discriminate ENPs from NNPs based on multi-element fingerprints. This approach can also be used for distinguishing Ti NPs types because TiO_2 ENPs are predicted to be purer than natural Ti-bearing particles. However, for some Ti-NNP types, the mass fraction of minor elements is low (< 0.01%), which causes small Ti-NNPs to be registered as single-element NPs (i.e. Ti- only) in spICP-TOFMS analysis. To avoid classifying small Ti-NNPs as Ti-ENPs, we must establish how much Ti signal needs be to recorded to also have a measurable secondary element (e.g. niobium). This particle-type detection limit can be used as a threshold to identify true single-element Ti-ENP signals.²

In this study, we investigate the classification of Ti-ENPs (TiO_2) in mixtures of Ti-containing NNPs (biotite, ilmenite and rutile) using spICP-TOFMS. From neat suspensions of NNPs, we find that we can correctly classify 31% of the rutile NNPs with 2.5% false ENPs, 73% of the ilmenite NNPs with 0.2% false ENPs, and 78% of the biotite NNPs with 0% false ENP classifications. For food-grade E-171 TiO_2 ENPs, we can classify 51% of the ENPs by particle number with no false NNP classifications. The fraction of particles classified for any particle type depends on the particle size

distribution and the particle-type detection limit. We will present details of our classification strategy and demonstrate classification of mixed ENP and NNP with number concentrations across three orders of magnitude.

1. Rihane, Naima, et al. Environmental Science and Pollution Research (2016)
2. Szakas, Sarah E., et al. Environmental Science: Nano (2022).

(Mon-P34) Laser Ablation Mass Spectrometry for Interrogating Nuclear Materials

Peter S. Boone¹, William Mason², Peter Hosemann², David Weisz¹, Brett H. Isselhardt¹; ¹*Lawrence Livermore National Laboratory*, ²*University of California, Berkeley*

Actinide isotope ratios in nuclear materials are important to post-irradiation examination (PIE) of spent nuclear fuel in both normal and failure modes, as well as forensic analysis of pre- and post-detonation nuclear materials. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used for spatially resolved measurements of isotope ratios and concentrations in five samples of sintered depleted UO₂. PIE and forensic efforts are complemented by information about a material's process history, which can be investigated through sample morphology and trace element analysis. Using LA-ICP-MS and SEM/EDS we characterized near-surface inclusions of W, confirming our ability to detect certain inhomogeneities that may aid in the understanding of a material's origin and history. The measured ¹⁸²W/¹⁸³W ratio was within approximately 1% of the naturally occurring value, while total W concentration was measured to within 6% of the expected value. This work aims to validate our approach as a high throughput method for measuring solid samples with minimal preparation. With data collection taking only minutes, samples can be quickly studied and flagged for further analysis.

(Mon-P35) Semi-supervised Machine Learning to Classify Cerium Nanoparticles Measured with spICP-TOFMS

Raven Buckman¹, Sarah E. Szakas¹, Alexander Gundlach-Graham¹; ¹*Iowa State University*

Nanoparticles (NPs) from various sources can be found in consumer products, industrial applications, and environmental samples. These NPs can be from anthropogenic or natural sources, and so being able to detect and quantify anthropogenic particles is critical for pollution sourcing determining toxicological impact. Cerium containing nanoparticles (Ce-NPs), in particular, are of interest due to their environmental prevalence in soil and water samples.

Previous studies have used both supervised and unsupervised machine learning algorithms to classify NP data from spICP-TOFMS. We propose utilizing a combination of both learning approaches, denoted as semi-supervised machine learning (S-SML), to classify Ce-NPs. S-SML requires both a small labeled and a large unlabeled training set; the unlabeled data is first clustered using an unsupervised machine learning algorithm and subsequently labeled similarly to the labeled data set. This combination of learning types does not require a complete manual labeling of the training set, as supervised learning approaches require, and is not limited to well-defined relationships, as unsupervised learning methods require.

In this study, we apply a S-SML algorithm to classify CeO₂, ferrocium mischmetal, and bastnaesite mineral samples as engineered (ENPs), incidental (INPs) or natural nanoparticles (NNPs), respectively. The training set is composed of randomly selected particle events from pristine samples of ENPs, INPs and NNPs, and, a second training cycle is used to create a fourth class of unclassifiable

nanoparticles (UNPs). Mixtures of ENPs, INPs and NNPs are classified using the S-SML model and compared to a statistical classification method. Simulation data was generated based on particle-type specific parameters to explore the advantages and limitations of using a S-SML modelling approach. We find that machine learning does not perform well when sensitivities fluctuate between experiments performed on different days. Additionally, correct classification of NPs is directly related to particle size; as the particle size becomes larger, the likelihood of the particle being misclassified decreases. If a particle mass is close to the limit of detection, the likelihood of misclassification increases drastically. Based on these results, for best classification, testing data and training data should be acquired at the same sensitivity and the particles should be sufficiently large.

(Mon-P36) Automated GC-MS analysis by KnowItAll MS Expert

Karl Nedwed¹, Ty Abshear¹, Michelle D'Souza¹, O. David Sparkman², James Little³; ¹Wiley Science Solutions, ²University of the Pacific, ³Mass Spec Interpretation Services

High resolution GC-MS data are information rich. Analysis can be time consuming, especially when examining complex analytes. We present a new expert system that combines fast, flexible automated deconvolution, combined with automatic database search to identify knowns and unknowns. Novel compounds can be identified, and structural components deduced from applying MS Adaptive search that applies fragmentation and structural data to propose likely structural details.

Monday Poster Session - PAT

(Mon-P37) Non-Invasive In-Line Raman Spectroscopy Enables Readiness for Flexible Bioprocess Monitoring

Christian Ott¹, Karin Wieland², Kristina Gruber¹, Christoph Haisch³, Thomas Brück³; ¹Schott AG, ²Competence Center CHASE GmbH, ³Technical University of Munich

This poster will introduce a comprehensive process analytical technology (PAT) solution with Raman spectroscopy and novel sensor interfaces. It enables enhanced automation and real-time in-line monitoring of bioprocesses without compromising the sterile boundary. The solution's readiness for industrial application is described by the use case of a pilot-scale yeast fermentation and its exemplary results of the chemometric data analysis for intracellular lipid production. The proposed set-up includes a novel sensor interface, which allowed higher flexibility during a running process due to flexibly changeable spectrometers and reduced overall contamination risk. The poster illustrates the experimental set-up and assembly and shows how the information derived from the spectra can be used for direct process control. The integration of the advanced PAT solution enables pharmaceutical and biotech companies to:

- Prevent contamination and optimize yield by measuring relevant chemical and physical parameters in-situ through a hermetically sealed optical window without the need to open the bioreactor for sampling, even under challenging process conditions such as harsh environments or sensitive organisms.
- Enable real-time process control and automation by capturing more physical and chemical process parameters and data, for example, of saccharide, peptide and cell growth. Deviations in the process flow can be identified in real time and immediately adjusted.
- Flexibly and efficiently integrate sensors into bioprocesses by enabling exchange and calibration during running process. The novel sensor interface is made of approved materials and can be sterilized in accordance with industry's best practices

In addition to pharma and biotech companies, the poster will be of interest to sensor and bioreactor manufacturers that want to further develop Raman spectroscopy and automation of bioprocesses.

(Mon-P38) Automated Sampling in Upstream Process Development for Accelerated Access to Critical Process Parameters and Critical Quality Attributes

Lee LEE Asplund¹, Srijana Chapagain¹, Rakesh Bobbala¹, Khin Myint¹, Stacy Shollenberger¹, Allyson Caron¹; ¹*MilliporeSigma*

To efficiently develop and deliver affordable biologics to patients, biopharmaceutical manufacturers are focused on accelerating timelines while improving quality standards and reducing costs over the lifecycle of their products. This relies on a shift from processes with primarily manual operations to a more automated workflow, enabling improved product quality and real-time batch release in production facilities as well as faster decision making in the development phases. Innovations in process analytical technologies (PAT) are helping to achieve this goal by providing more comprehensive monitoring and control capabilities, and earlier information about the product and process. PAT allows for measurement of critical process parameters (CPPs), which are essential to maintain within a specified range to ensure reproducible control of the process, and critical quality attributes (CQAs), which provide information about the resulting product quality. Historically, offline analytical technologies have required a significant manual burden in which operators obtain, process, and assess a sample of interest which can take days or weeks to complete. To address this challenge in upstream cell culture processes, an enabling technology has emerged to provide automated, aseptic sampling. Automated sampling systems can take a sample from the bioreactor while maintaining its sterility and deliver the sample to a variety of analytical instruments for real-time analysis, without the need for human intervention. In perfusion cell culture modes where processes run continuously, this is especially important to ensure quality across the entire run duration and take corrective actions to prevent deviations. This poster will focus on the MAST[®] automated sampling system and its utilization in perfusion cell culture for accelerated upstream process development. We will demonstrate increased frequency of data acquisition for online CPP measurement and automation of a sample processing workflow to reduce the time to CQA measurement in perfusion cultures. Additionally, we will discuss the value of combining automated sampling with Raman spectroscopy for increased sample frequency during chemometric model building, leading to more accurate inline and real-time measurement of CPPs and CQAs in the upstream bioprocess.

(Mon-P39) On Digital Bioprocessing for manufacturing intelligence: Application of Process Analytical Technology (PAT) and Process Data Analytics (PDA) for upstream process development and intensification

Ricardo Suarez Heredia¹, Marina Hincapie¹, Kevin Brower¹, Henry Lin¹, Nihal Tugcu¹; ¹*Sanofi*

Background: Digital bioprocessing has greatly benefited from ongoing developments in advanced sensor technology, robotic high throughput experimental platforms, consolidation of a broad range of analytical techniques, increased computational power and data management systems. Therefore, the steady integration of model-based and data analytics tools to analyze, interpret and use complex real-time and historical data repositories can lead to improve operational efficiency and robustness. Herein, we introduce three case studies to exemplify some of the applications of digital bioprocessing for descriptive, diagnostic, predictive and prescriptive support for upstream process development by combining principles of process analytical technology (PAT) and process data analytics (PDA).

Methodology: The first case study introduces a PAT framework for the development and GMP-ready implementation of Raman spectroscopy soft sensors for in-line and real-time monitoring and control of critical process parameters (CPP) in mammalian cell culture-based manufacturing. The second case study introduces the development of a PDA pipeline for the predictive and historical investigation of the impact of process strategies on growth and productivity of historical mammalian cell cultures (>120 cultures). The third case study introduces the use of evolutionary computation, particularly

genetic algorithms and multigene genetic programming, in combination with automated microbioreactors (ambr®15) for the rapid process design and the intensification of mammalian cell cultures and as a heuristic alternative to the statistical design of experiments approach.

Results and discussion: The first case demonstrated the implementation of a multidisciplinary PAT approach for data pre-processing and processing of time series Raman spectroscopy for in-line process monitoring. The second case highlighted the implementation of data pre-processing and processing methods for a supervised cross modelling approach used to predict the harvest titer based on other process features as early as exponential phase as well as identifying feeding strategies (regime and composition) leading to improved productivity. The third case study resulted in an accelerated process design screening (perfusion media composition and regime), sensitivity analysis and process improvement based on multi-objective optimization for dynamic perfusion cultures.

Overall, these case studies introduce the potential role of digital bioprocessing for manufacturing intelligence in next-gen bioprocessing platforms.

(Mon-P40) **Carbon Dioxide Species In Tetramethylammonium Hydroxide Systems Using Macroscopic Raman Spectroscopy**

Michelle N. Sestak¹, Timothy M. Holt¹; ¹*HORIBA Instruments Incorporated*

Tetramethylammonium hydroxide (TMAH), a strong base (pH ~13), is commonly used in the semiconductor industry as part of a developer or silicon etching solution. It is well known that TMAH absorbs CO₂ from the environment and forms carbonates. pH measurements are a well established means for distinguishing between carbonate and bicarbonate ions that are formed. However, incorporating other materials into the solutions that buffer the pH in the same range of bicarbonate/carbonate, can confound the implied speciation, making pH an ineffective technique for distinguishing between carbonate and bicarbonate ions in chemical mixtures and blends. We present macro-Raman spectroscopy as an alternate means for distinguishing between carbonate and bicarbonate ions present in various TMAH solutions. Macroscopic Raman spectroscopy is an ideal technique because it allows quick, easy in-line measurements, or off-line measurements through sealed bottles, which is a safe and effective method for measurement of toxic solutions, such as those containing TMAH. This method may also be expanded for other applications where carbonate/bicarbonate speciation is important, such as in Earth and life sciences.

Monday Poster Session - PMA

(Mon-P41) **TD-NMR of Albumin Sources**

Gregory K. Webster¹, Steven Doherty¹; ¹*AbbVie*

TD-NMR has been successfully implemented with identity testing applications, particularly within the food industry. For proteins, the technique analyzes fast chemical exchange between water and exposed NH and OH protons of amino acid side chains in the folded protein structure unique to each biologic. Analysis of dominant features in folded proteins in solution can be exploited for other types of biopharma assets as well as for authentication, forensics, and supply chain integrity where other spectroscopic or chromatographic testing techniques cannot detect a difference. In addition, TD-NMR has been used to determine the water content of lyophilized proteins and the aggregation of proteins in solution. For small molecule applications, TD-NMR can detect if solvents are received neat or tainted with moisture, impurities, or denaturants.

The purpose of this study was to evaluate the ability of time-domain NMR (TD-NMR) to differentiate between sources of Albumin proteins as a rapid QC test. Evaluation of differences in molecular mobility between components in a solution as reflected in the longitudinal (T_1) and transverse (T_2) relaxation times of protons demonstrate that TD-NMR techniques can distinguish between different Albumin sources.

(Mon-P42) Recent Trends in Active Pharmaceutical Ingredient Profiles of Counterfeit Alprazolam Tablets

Melanie N. Parsons¹, Enrique Yanes¹, Kelsey Griffin², Mary Jones¹, Valerie Toomey², Skyler W. Smith¹, Flavia Morales-Garcia¹; ¹*U.S. Food & Drug Administration*, ²*US Food and Drug Administration*

The Forensic Chemistry Center (FCC) of the U.S. Food & Drug Administration (FDA) provides analytical support to the FDA Office of Criminal Investigations (OCI). As such, FCC receives submissions of suspected counterfeit drug products, including suspect counterfeit alprazolam tablets. The tablets have the appearance of legitimate products. While alprazolam is identified in many submissions; an increasing number have had one or more other active pharmaceutical ingredients (APIs) identified. The presence of undeclared APIs may pose significant risk to public health. The other APIs identified represent the following drug classes: benzodiazepine derivative, thienotriazolodiazepine, tricyclic antidepressant, tryptamine, antihistamine, amphetamine, antiviral and unknowns. One concern is that the amphetamines and tryptamine could produce amphetamine-like stimulation or hallucinogenic effects. Another concern is lack of FDA approval for many of the identified APIs. The APIs were identified using gas chromatography and/or liquid chromatography with mass spectral detection (GC-MS or LC-MS). Tablet composites were extracted and diluted prior to instrumental analysis. GC-MS analyses were performed using a temperature gradient with a Phenomenex Zebron ZB-5MSi or ZB-5HT capillary column with a run time of 33.5 minutes. LC-MSⁿ analyses were performed using ultra high performance liquid chromatography (UHPLC) systems coupled to ion-trap mass spectrometers with an electrospray ionization source. The LC-MS methods utilized a gradient with 0.1% formic acid in water, 0.1% formic acid in acetonitrile and C18 columns to separate the compounds. High performance liquid chromatography with ultraviolet detection (HPLC-UV) analyses were performed to determine the amounts for seven selected APIs from seven different samples, four of which had multiple APIs. HPLC-UV instrument parameters were dependent on the actives identified. Concentrations of individual APIs ranged from 0.089 mg/tablet to 1.36 mg/tablet. Here we report some of the recent trends observed during analysis of counterfeit alprazolam tablets.

(Mon-P43) Continuous Mixing Technology: Characterization of a Vertical Mixer Using Residence Time Distribution

James Kimber¹, Kai Lee¹, Giuseppe Cogoni¹, Jenna Brandon¹, David Wilsdon¹, Hugh Verrier¹, Sally Grieb¹, Ashwinkumar Jain¹, Pankaj Doshi¹, Daniel Blackwood¹; ¹*Pfizer*

Continuous powder mixing technology application during continuous direct compression process has emerged as a leading technology used in the development and manufacture of solid oral dosage forms. The critical quality attributes (CQAs) of the final product are heavily dependent on the performance of the mixing step as the quality of mixing will directly influences the content uniformity of the tablet.

Therefore, the aim of this study was to investigate the impact of blend properties (bulk density, API sizes) and process parameters (process throughput, hold up mass and impeller speed) on the mixing performance. Mixing performance was characterized using the residence time distribution (RTD) of the process, which has been broadly used to characterize unit operations in pharmaceutical processes. In this work, the RTDs for a vertical continuous mixing device termed Continuous Mixing Technology (CMT) were obtained in order to evaluate mixing performance over a defined operating space. The RTDs were determined using a spike injection of tracer material into the mixer and measuring the concentration of the tracer in the outgoing material. This was achieved using a PAT interface situated at the exit of the CMT that presents the material to a near-infrared (NIR) probe. The theoretical residence time of the mixer is given by the hold-up mass divided by the throughput, and for each experiment, NIR spectra of the exiting material was measured for five theoretical residence times after tracer injection to obtain the full RTD.

As the CMT contains an upper de-lumping screen and a lower mixing chamber, the residence time distributions of this system can be described as two CSTRs in series with different residence times where the parameter 'r' describes the ratio between these residence times. The best value of 'r' for all experiments was determined through simultaneous optimization of all measured RTDs. The results showed that the CMT operates close to a single CSTR over the whole operating space, has good ability to dampen the fluctuations from the gravimetric feeders, and that the mixing performance of the CMT is not significantly impacted by blend properties (i.e. bulk density and API particle size) [<https://doi.org/10.1016/j.xphs.2021.01.035>].

(Mon-P45) Comparison of Raman and Near-Infrared Chemical Imaging for Analysis of 3D Printed Formulations

*Zoë Whalley*¹, Patrick Wray², Tom Mills¹, Richard Greenwood¹; ¹*The University of Birmingham*, ²*Bristol Myers Squibb*

3D printed pharmaceuticals are an emerging field, with potential applications for personalised medicine and modified drug release. Increased manufacturing understanding will help to improve the quality of the final product, allowing the technology to benefit patients sooner.

Vibrational spectroscopic imaging and mapping techniques such as Raman and Near-Infrared (NIR) allows us to non-destructively obtain spatially-resolved chemical information about the distribution of components within a 3D printed pharmaceutical formulation.

The dosage forms evaluated in this study consisted of a 50:50 HPMC (Hydroxypropyl methylcellulose):HPC (Hydroxypropyl cellulose) SL polymer mixture with various drug loadings of caffeine. Tablets of different caffeine concentrations were printed and analysed using Raman and Near-Infrared mapping techniques. Component distributions were discriminated through use of a PLS (Partial Least Squares) model based on pure component spectra. The resulting information was used to understand the relationship between the formulation, the processing steps of hot melt extrusion and printing and how they affect the final 3D printed tablet.

Near-Infrared imaging has a faster acquisition time relative to Raman techniques. Raman better discriminated domains of components, especially within low regions of crystalline material, which were indiscernible on the equivalent NIR image. This work demonstrates both techniques are viable for discerning components in 3D-printed tablets.

Monday Poster Session - SPECIAL

(Mon-P46) **Characterizing Aromaticity of Triplet Corannulene and Coronene**

Dmitrii Govorov¹, Nirroodha R. Pitawela¹, Anna Gudmundsdottir¹; ¹*University of Cincinnati*

Aromaticity is a property resulting from cyclic π -conjugation or electron delocalization in closed circuits, either in two or three dimensions. To date, there is no precise definition of aromaticity accepted by the scientific community as a whole.

The Hückel's rule relates the number of π -electrons with the aromaticity of a molecule. The photochemistry analogue of Hückel's rule, known as Baird's rule, can be applied to the lowest triplet and excited singlet states and predicts $4N$ π -electron molecules to be aromatic and $4N + 2$ molecules to be antiaromatic. According to these rules, the corannulene molecule in its singlet ground electronic state (S_0) is conditionally considered as antiaromatic and aromatic in the triplet electronic state. Similarly, the coronene molecule is antiaromatic in the S_0 state and aromatic in the T_1 state.

Nucleus-independent chemical shifts (NICS) method was used to analyze the magnetic properties of corannulene and coronene molecules in conjunction with the analysis of anisotropy of the magnetically induced current density (ACID).

Our study contradicts the Hückel's and Baird's rules as both molecules were found to be antiaromatic in the T_1 state. Having very similar structures, these molecules have slightly different magnetic properties as can be seen from the NICS and ACID analyses we presented. Since the coronene molecule is planar, it is stronger π -conjugated than corannulene in both S_0 and T_1 electronic states. This property allows it to produce stronger circular currents in the presence of magnetic fields, although, in the triplet state the circular currents in both of these molecules split into distinct center and rim domains. This bifurcation becomes especially pronounced in the T_1 electronic state, accompanied with the merge of the circular current directions.

(Mon-P47) **Photofracking of 1-Azido-2-Nitrobenzene Crystals**

Brandi James¹, Kristine Maxwell², Anna Gudmundsdottir¹; ¹*University of Cincinnati*, ²*Truman State University*

Photodynamic organic crystals have potential as applications for benign devices including actuators and sensors. More specifically, gas release crystals are interesting due to their potential application towards making safer explosives and selectivity of the reactions. We are studying the photoreactivity of crystalline azido compounds to identify how the release of nitrogen gas within the crystals lattice affects their photodynamic behavior.

Herein, we will present and discuss the photo-response of 1-azido-2-nitrobenzene (**1**) derivatives upon irradiation. This concerted reaction of **1** to form benzofuroxan has been extensively studied in previous literature using time-resolved studies in solution, but minimal studies in the solid-state. Energy framework calculations of the crystal lattices were completed to understand where the crystals are most likely to fall apart. We will correlate the effects of external pressure towards the photodynamic behavior of these organic azide crystals.

Tuesday, October 4, 2022

Oral Presentations

22AES02: Electrokinetic Fundamentals

Chair: Rodrigo Martinez-Duarte

(AES-02.1) **High Sensitivity in Dielectrophoresis Separations**

Benjamin G. Hawkins¹, Benjamin G. Hawkins¹; ¹*Cal Poly, San Luis Obispo*

While the use of dielectrophoresis (DEP) techniques for the cell and particle trapping or capture are well-established. However, the robust techniques for manipulation of cells in a label-free fashion within microfluidic systems to enable highly sensitive separations remain elusive. The ability of DEP to isolate subtle phenotypic differences within a population of cells remains unrealized. We consider notable efforts to leverage that most compelling aspect of DEP – its dependence on specific particle electrical properties – while overcoming the confounding effects of phenotypic variations in cell size. These techniques, centering around the application of multiple electric fields with spatially mapped magnitude and/or frequencies, have the potential to expand the capability of DEP cell separation.

(AES-02.2) The synthesis of Bacterial Cellulose under AC electric fields

Rodrigo Martinez-Duarte¹, Sindora R. Baddam¹; ¹*Clemson University*

Assessing potential to use *K. xylinus* as a robotic printer of nanocellulose scaffolds.

We present initial results on using electric field gradients, in a technique called dielectrophoresis (DEP), for the spatiotemporal manipulation of the bacterium *K. xylinus*; and the impact of the electric field on the synthesis of bacterial cellulose (BC). These are crucial steps for the use of *K. xylinus* as a robotic printer of architected nanocellulose scaffolds that can be used as is or serve as a precursor to multiple carbonaceous materials.

K. xylinus can synthesize BC, an advantageous source of cellulose over plant cellulose in terms of strength and purity. Particularly, the extraction of biomass from plants and purification of cellulose to remove hemicellulose and lignin is not required in BC production. The use of a bioreactor to produce BC also eliminates the need for forestry operations to produce cellulose. While the fabrication throughput of BC may appear low, it must be compared to the growth rate of plants, with the advantage that pure BC can be directly obtained. By combining DEP techniques with *K. xylinus*, we attempt to architect BC from the bottom-up and create engineered films that can be subsequently shaped using film shaping techniques like folding and origami.

We present two major developments towards the use of *K. xylinus* as a tiny printer: 1) the use of light-induced DEP, or the use of light fields to enable an electric field gradient, to enable basic manipulation of *K. xylinus* cells; and 2) the use of a microfluidic reactor featuring microelectrodes to study how electrostimulation can potentially manipulate and influence the synthesis of BC. This second study features experiments running over fourteen days with an AC induced electric field at polarization voltages of 1 V_{pp}, 2 V_{pp}, and 5 V_{pp} at a frequency of 750 kHz. Results suggest that long-term BC synthesis is possible under electric fields necessary for *K. xylinus* manipulation.

(AES-02.3) **Particle Properties Influence on the Electrokinetic Equilibrium Condition and Nonlinear Electrophoretic Mobility**

Olivia Ernst¹, Curran Dillis¹, Adrian Lomeli-Martin¹, Blanca H. Lapizco-Encinas¹;

¹*Rochester Institute of Technology*

First report on the influence of particle properties on the electrokinetic equilibrium condition

The Electrokinetic Equilibrium Condition (E_{EEC}), a condition where all EK effects exerted on the particle are in equilibrium, can be found experimentally via particle image velocimetry. The methodology for estimating the E_{EEC} of particles is still new. Although there have been hints of a relationship between particle size and E_{EEC} magnitude, no study has confirmed this relationship. The present research study aims to answer these questions by investigating the potential correlation between E_{EEC} , particle size, and particle charge (particle zeta potential). The main goal is to define a mathematical prediction of the E_{EEC} and nonlinear electrokinetic mobility ($\mu_{EP}^{(3)}$) for individual particles as a function of particle properties (size and charge). This prediction could be an alternative means of estimating these values, requiring less experimental time and effort than current methods. The relative simplicity of this alternative process would expedite and simplify particle characterization. The data obtained from this project can be utilized to design electrokinetic separation processes of microparticles and microorganisms.

Acknowledgments:

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(AES-02.4) **MOF-based Janus Micromotor Locomotive Characterization**

Eric R. Languirand¹, Matthew Collins²; ¹*U.S. Army DEVCOM CBC*, ²*Leidos*

MOF-based Janus micromotors may be a useful tool for chemical decontamination and characterization is necessary.

Synthetic self-powered autonomous nano/micromotors are a class of active systems that have been used in pollution mitigation, self-assembly, chemical sensing, and targeted drug delivery. Additionally, chemical decontamination has also been explored as a possible avenue for use of this type of active system. Specifically, active systems provide movement beyond that of Brownian motion, effectively increasing the rate of mixing and therefore possible chemical reaction. Typical motors are asymmetrical particles that catalyze a chemical reaction by generating either a proton gradient or bubbles upon addition of hydrogen peroxide as fuel. In addition, metal-organic-frameworks (MOFs) have been added to micromotor systems in an effort to increase water purification/nuclear waste removal. Therefore, MOF-based Janus micromotors have the potential for use in the rapid and efficient decontamination of chemicals.

Herein, we present work towards the locomotive characterization of MOF-based Janus micromotors. This work utilizes spherical Janus micromotors whereby the speed and mean

square displacement of the micromotors are characterized under different conditions. We explore the use of different metals (Ag, Pt, and CsO₂) to hemispherically coat micromotors of two sizes (3μm and 20μm). Furthermore, we look into the effects of differing amounts of fuel (H₂O₂) on the speed and mean square displacement of the micromotors. We determine the characteristics of the micromotor movement based on the different combinations of metal, fuel, and size as well as showing differences between MOF-coated and bare polystyrene micromotors to help inform an ideal choice for chemical decontamination.

(AES-02.5) **Methodology for characterizing the Nonlinear Electrokinetic Behavior of Microparticles**

Adrian Lomeli-Martin¹, Olivia Ernst¹, Richard Cobos Franco, Aditya Khair², Blanca H. Lapizco-Encinas¹; ¹*Rochester Institute of Technology*, ²*Carnegie Mellon University*

We present a new strategy for characterizing the nonlinear electrokinetic behavior of particles and cells

Recent developments in the field of insulator-based electrokinetics (iEK) have illustrated the importance of the nonlinear EK phenomenon of electrophoresis of the second kind (EP⁽³⁾). Thus, it is essential to develop a robust methodology for characterizing the EP⁽³⁾ mobility (μEP⁽³⁾) of microparticles/microorganisms. A partial methodology has been reported that is only applicable if the analyzed particle fulfills the following criteria: (i) particle charge has the same sign as the channel wall charge, and (ii) the magnitude of the particle zeta potential is lower than that the zeta potential of the channel wall. No methodology has been developed to characterize the particle μEP⁽³⁾ when the analyzed particle has either a zeta potential greater in magnitude than the channel wall or the particle has an electrical charge of the opposite sign of that of the channel wall. This project aims to fill this gap by developing a methodology to enable the characterization of the μEP⁽³⁾ of all types of microparticles providing a generalized procedure that can be applied to microparticles and even microorganisms.

Acknowledgments:

This material is based upon work supported by the National Science Foundation under Award No. 2127592.

22ATOM07: ICP-MS Applications

Chair: Jenny Nelson

(ATOM-07.1) **Elemental Analysis of Kratom Products and E-Liquids Samples using ICP-MS**

Madhavi Mantha¹, Kevin Kubachka¹, Robert Wilson; ¹*US Food and Drug Administration*

US FDA's Forensic Chemistry Center (FCC) receives different kinds of samples for forensic analysis, often without defined methods. Two relatively recent projects were the 2018 kratom product survey and 2019 E-Liquid Vaping Associated Lung Injury (EVALI) crisis samples.

In 2018, FCC received 26 different unapproved kratom products with unsubstantiated claims about treating serious medical conditions. The lack of validated methods for screening of heavy metals in kratom-related products resulted in significant challenges due to the complex and diverse sample matrices. Faced with little information about the levels of concern for metals in kratom products, FDA Elemental Analysis Manual Method (EAM 4.7) was modified to fit the purpose of analysis and used Q3D Elemental Impurities Guidance to determine reporting limits. The results from the analysis indicated high levels of heavy metals, including V, Cr, Co, Ni, As, and Pb in nearly all the samples tested. Hazardous levels of Pb and Ni were identified in several samples at concentrations that exceed safe exposure for oral daily intake based on a toxicological evaluation conducted by CDER, which accounted for kratom usage patterns and the safe daily exposure limits found in the Q3D guidelines.¹

The second part of the presentation details work from 2019 EVALI crisis. FCC received vaping devices from various states and other sources. One of the many examinations looked at the element contaminations of the nicotine and tetrahydrocannabinol (THC) e-liquids. Due to its hydrophobicity, extremely high viscosity, and adhesion compared to nicotine, THC based e-liquids proved difficult to work with and were further complicated by the low amount of sample received

1. <https://www.fda.gov/news-events/public-health-focus/laboratory-analysis-kratom-products-heavy-metals>

(ATOM-07.2) **Metallomics to Study Cancer Metabolism in Clear Cell Renal Cell Carcinoma**

Julio Landero¹, Julio Landero¹, Dina Secic², Maria Czyzyk-Krzeska², James Reigle², Behrouz Shamsaei², Mario Medvedovic², David Plas², Tom Cunningham², Jarek Meller², Shuchi Gulati²; ¹*Icahn School of Medicine at Mount Sinai*, ²*University of Cincinnati*

Clear cell renal cell carcinoma (ccRCC) is a frequent and malignant renal cancer commonly characterized by elevated aerobic glycolysis. Tobacco smoking is a known risk factor for ccRCC, with elevated toxic metals associated, but the molecular mechanisms involved are not well understood. The first line treatment of localized disease is surgical removal of the tumor. However, up to 50% of patients relapse, there are no biomarkers for risk of recurrence. Therefore, there is an urgent scientific and clinical need to understand the molecular mechanisms leading to the relapse, establish prognostic biomarkers, and to improve adjuvant treatment strategies.

The impact of tobacco smoking on the metabolic phenotype of ccRCC, and changes in survival rates associated was the central topic of this research. The metabolic phenotype of ccRCC primary tumors of never smokers (NS) and lifetime smokers (LTS) was studied by gene expression analysis, LC-MS/MS based metabolomics and metallomics.

The tumors from LTS contained more Cu, As and Cd than NS. The presence of high and low molecular mass Cd fractions in tumors from LTS was in sharp contrast to the metallothioneine (MT) bound Cd, and no low molecular mass Cd in NS. The arsenic

speciation revealed an increase in inorganic As from 25% in NS to over 60% in LTS, with concomitant decrease in S-adenosyl methionine. Copper increased in LTS tumors, and the proteome distribution showed a redistribution from MT-Cu to a high molecular mass fraction identified as cytochrome-C oxidase (Cu-COX).

Major metabolic differences were observed between NS and LTS tumors, that indicate a shift from highly glycolytic to oxidative phosphorylation based ATP production. The metabolic reprogramming was detected by metabolomics gene expression, and allowed for a survival analysis classification.

The Cu-COX signal defined from SEC-ICP-MS was validated as a marker of functional cellular respiration in cultured kidney cancer cell lines, with direct correlation to oxygen consumption rates (OCR) under galactose or elevated copper culturing conditions. The use of this new biomarker is proposed as a new tool for metabolic phenotyping of cRCC tumors with future prognostics value.

(ATOM-07.3) Determination of Minerals and Trace Elements in Milk, Milk Products, Infant Formula, and Adult/Pediatric Nutritional Formula by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Lawrence H. Pacquette¹, Lawrence H. Pacquette¹; ¹*Abbott Nutrition*

Determination of Minerals and Trace Elements in Milk, Milk Products, Infant Formula, and Adult/Pediatric Nutritional Formula by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Lawrence Pacquette

Abbott Nutrition – 3300 Stelzer Road, Columbus, OH 43219, United States

This method, AOAC Method 2016.05, is an in-house validated method used Abbott Nutrition for the determination of Na, Mg, P, K, Ca, Cr, Mn, Fe, Cu, Zn, Se and Mo in Milk, Milk Products, Infant Formula, and Adult/Pediatric Nutritional Formula by ICP-MS. It was developed in response to a need for modern reference methods for dispute resolution. The method first gained AOAC First Action status by meeting all standard method performance requirements (SMPRs) developed by the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) working group. The next step was to validate the method for precision and reproducibility, through multi-lab testing (MLT), to determine its suitability for Final Action and dispute resolution status. Both reproducibility and suitability were primarily determined by how well the SMPR was met during a collaborative MLT study by a minimum of eight laboratories using different models of ICP-MS instruments. An additional component of the study was to gather similar data on dairy products for the International Dairy Federation (IDF), who were looking for official methods for the determination of minerals in common dairy ingredients, milk, and milk powder. A concurrent MLT study was also performed with the same samples (in different labs) and nearly identical protocol using a similar methodology, inductively coupled plasma atomic emission spectrometry (ICP-AES), based upon AOAC Official Method 2011.14 and ISO

15151 I IDF 229. Comparable results were obtained between the ICP-MS and the ICP-OES methods. However, due to the lack of sensitivity of the ICP-AES method the ultra trace elements (Cr, Se, and Mo) were not measured. There was substantial evidence to support the accuracy and reproducibility of this method through comparison to an independent method and through analyses completed at independent laboratories with different models of ICP-MS instruments.

(ATOM-07.4) Characterization of Silicon Dioxide Food Additives via Single Particle Inductively Coupled Plasma Mass Spectrometry and Other Techniques

Monique E. Johnson¹, Sadia Khan², Antonio R. Montoro Bustos¹, Karen E. Murphy¹, Michael Winchester¹, Timothy Croley², Ingo H. Streng³, Savelas Rabb¹; ¹*National Institute of Standards and Technology*, ²*Food and Drug Administration*, ³*University of Siegen*

We present the analysis of 6 SiO₂ food additive materials by single particle ICP-MS

Silicon dioxide (SiO₂), in its amorphous form, is an approved food additive (21 CFR 172.480) in the U.S., mainly used as an anticaking agent in powdered food products and as stabilizer in the production of beer. During production, there is a possibility of nanoscale silicon dioxide particles to occur in food additives as well as the potential for primary particles to aggregate to form nano-sized and/or larger particles. However, there is currently limited data concerning the particle size distributions of food grade SiO₂. To address this issue, FDA's Center for Food Safety and Applied Nutrition (CFSAN) began collaborative work in 2019 with the Inorganic Chemical Metrology Group at the National Institute of Standards and Technology (NIST) to develop reliable analytical methods for the characterization of metal oxide nanoparticles in food additive materials. This work describes the characterization of six commercially available food grade SiO₂ powders that were prepared for analysis using four different sample preparation procedures (shaking, sonication, shaking+filtration, and sonication+filtration). Total Si mass content was determined by inductively coupled plasma optical emission spectrometry, while size characterizations were carried out using dynamic light scattering, scanning electron microscopy, and single particle inductively coupled plasma mass spectrometry (spICP-MS). In-house characterized commercially available SiO₂ nanoparticles served as calibration materials for the determination of total mass fractions and size distributions (to aid spICP-MS measurements), across all sample preparations. SiO₂ food additive material samples that underwent a filtration step exhibited a mass fraction reduction of over 90 %. The sample preparation procedures resulted in suspensions containing particles in the range of 300 nm to 500 nm. Overall, this study will highlight the benefits of spICP-MS analysis as a size characterization method, capable of providing insights in the area of food safety and regulation.

(ATOM-07.5) Advanced Trace Element Analysis using Plasma Spectroscopy in Environmental Monitoring (Fine Dust, Virus, and Toxic Gas)

Jun-Ho Yang¹, Jack Yoh¹; ¹*Seoul National University*

3-channel SIPS device is designed for detecting fine dust, virus, and toxic gas

This paper deals with a new attempt and state-of-the-art study on environmental monitoring using spark-induced plasma spectroscopy.

Air pollution is increasing globally owing to rapid industrialization and modernization. With the rising environmental and health concerns for air quality, an advanced system for real-time monitoring of toxic components (fine dust, virus, and toxic gas) should be developed. Based on spark-induced plasma spectroscopy (SIPS), we present a compact all-in-one sensing module to identify the atomic components of fine dust in situ. Embedded software was used to integrate all the functional aspects of time-resolved electric signals, such as plasma creation, measurement, and data visualization into a single module. First, we validated the performance of the all-in-one module by quantitatively detecting the atomic components of fine dust, namely, Fe, Mg, Si, Ca, Al, and K, with high sensitivity (< 0.1 ppm). Then, the risk of air propagation was assessed in terms of changes in virus concentration concerning distance traveled and measurement time. Finally, the proposed SIPS is used for detecting toxic molecules (formaldehyde, acetaldehyde, acetic acid, toluene, and ammonia) in real-time (< 3 sec) at relatively high sensitivity (< 5 ppm). As a result, our research serves as a model for performing real-time virus detection and instantaneous monitoring of toxic components in the air.

22BIM05: Nanotheranostics: Diagnosis and Treatment of Disease using Nanomaterials

Chair: Samuel Mabbott

(BIM-05.1) Optimization of SORS Instrumentation for Applications in Preclinical Cancer Imaging

Fay Nicolson¹, Bohdan Andreiuk¹, Bridget O'Donnell², Eunah Lee², Andrew Whitley², Scott Rudder³, Kevin Haigis¹; ¹*Dana-Farber Cancer Institute and Harvard Medical School*, ²*HORIBA Scientific*, ³*OptoSigma*

Here, we combine the use of "spatially offset Raman spectroscopy" (SORS) with that of Surface Enhanced Raman Scattering (SERS) nanoparticles in a technique known as "surface enhanced spatially offset Raman spectroscopy" (SESORS) to image deep-seated tumors. We will discuss the optimization of SORS instrumentation and imaging approaches, and subsequent application of SESORS to pre-clinical cancer imaging and delineation of tumor margins in *Apc^{fl/+}*, *Apc^{fl/+};Kras^{G12D/+}*, and GL261 mouse models of colorectal cancer and glioblastoma respectively. We demonstrate that our approach enables advancements in the non-invasive detection of these cancers due to improvements in SNR, spectral resolution, and depth acquisition, and can complement clinically approved radiologic techniques.

(BIM-05.2) Wearable Plasmonic Paper-based Microfluidics for Sweat Analysis

Limei Tian¹, Limei Tian¹; ¹*Texas A&M University*

Wearable sweat sensors have the potential to provide valuable information related to the health and disease states of individuals. Existing sweat sensors mainly rely on

biomacromolecules, such as enzymes and antibodies, as biorecognition elements to achieve specific quantification of metabolites and hormones. However, these biomacromolecules tend to degrade over time, limiting the sensors' shelflife and compromising the sensor performance upon environmental changes, such as varying temperature and humidity. Here, we introduce a wearable plasmonic paper-based microfluidic system to continuously and simultaneously quantify sweat loss, sweat rate, and metabolites in sweat. Plasmonic sensors based on surface-enhanced Raman spectroscopy (SERS) are label-free and can identify the analytes of interest via the chemical "fingerprint" information. We show that simple and low-cost plasmonic papers allow for the sensitive detection and quantification of uric acid in sweat at a low concentration of 1 μ M. The well-defined flow kinetics of paper microfluidic devices enable accurate quantification of sweat loss and sweat rate in real time. Reliable quantification can be achieved when the devices are under strain and at high temperatures. We demonstrated two operation modes for continuous monitoring of UA at changing concentrations that are physiologically and pathologically relevant in human sweat. The wearable plasmonic device is soft, flexible, and stretchable, providing a robust interface with the skin without inducing chemical or physical irritation.

(BIM-05.3) Deep Raman Diagnosis: A Combined Role of SERS Nanostructure, Instrumentation and Ex Vivo Tissue Model

Priyanka Dey¹, William Olds², Alexandra Vaideanu³, Andreas Schatzlein³, Idriss Blakey⁴, Peter Fredericks², Pavel Matousek⁵, Nicholas Stone⁶; ¹*Teesside University*, ²*Queensland University of Technology*, ³*University College London*, ⁴*University of Queensland*, ⁵*STFC Rutherford Appleton Laboratory*, ⁶*University of Exeter*

Surgical tissue biopsies are still the gold standard for cancer diagnosis, which can be painful and often inconclusive. On the other hand, non-surgical diagnosis techniques are less painful and enable rapid treatments to be applied. They also offer the possibility of combining diagnosis and therapy - a step towards personalized medicine. Nevertheless, this requires custom-designed nano-imaging agents. When these are injected into the bloodstream, they can accumulate at targeted cancer sites. Specialized medical devices are then employed to track these agents and detect the location and type of cancer, aiding non-surgical cancer diagnosis.

Optical near-infrared (NIR) Raman spectroscopy offers unparalleled sensitivity for diagnosis and when appropriate optical systems are used, the significant tissue penetration of NIR photons allows for collection of scattered Raman photons from depths of many mm. However, for specific detection of deep lesions, strong and distinct signals are required to distinguish the tumour from the surrounding soft tissue. Attempts using Raman labelled plasmonic nanostructures benefitting from surface enhanced Raman scattering (SERS) with specialised subsurface detection methods like deep Raman spectroscopy (DRS) techniques are promising.

This presentation will discuss the importance of tailoring the SERS nanostructure imaging agent, as well as the DRS imaging optics to enable detection from deeper layers of animal tissue. A perfect union between them would allow maximization of the detection depths

with Raman spectroscopy- an avenue that is of great interest for cancer lesion detection in humans. Sub-100 nm gold nanostructures with high colloidal stability apt as injections will be discussed, including spherical nanoparticles of various sizes and nano-assemblies built from them. Advances in DRS instrumentation approaches will be discussed and compared to determine their benefits suitable for specific end uses. The journey of improvement of detection depth from less than 4 mm to 71 mm, will be discussed.

(BIM-05.4) Understanding the Intracellular Uptake of Nano-based Drug Delivery Systems in Cancer Therapeutics

Aristea Anna Leventi¹, Kharmen Billimoria², Dorota Bartczak², Stacey Laing¹, Heidi Goenaga-Infante², Karen Faulds³, Duncan Graham³; ¹*University of Strathclyde*, ²*NML at LGC*, ³*The University of Strathclyde*

New mechanistic insights for the internalisation of cisplatin-nanoparticle conjugates at an intracellular level. (SERS/SRS/LA-ICPMS analysis)

Cancer is the leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 alone. Treatment options usually include the use of chemotherapeutic agents that bind to DNA and induce cell death. One of the leading agents for systemic treatment of solid tumours is cis-diamminedichloroplatinum(II), otherwise known as cisplatin; however its clinical use is hindered by the toxic side effects attributed to the drug accumulation in both healthy and cancerous tissue. To overcome these challenges, nanoparticles can be adopted as drug delivery vehicles that can passively target solid tumors and minimize the uptake by healthy cells. Therefore, by incorporating a nano-based drug delivery system there is the potential to reduce the drug's toxicity.

In this study, we report the synthesis and characterization of gold nanoparticles as delivery vehicles for the antitumoral drug cisplatin. The resulting drug-conjugates were investigated for their stability, elemental composition, drug loading and interaction with cancer cells. Due to their composition, the drug-conjugates were amenable to multi-elemental detection by laser ablation inductively coupled plasma time of flight mass spectroscopy (LA-ICP-ToF-MS), which verified the spatial distribution and internalization of drugs in cells. For a detailed investigation of the fate of the drug within the cells, an alkyne-tagged approach was adopted, which provided a signature peak in the 'biologically silent' region (1800 – 2800 cm^{-1}) that is free from interference from other cell components. This allowed the intracellular tracking of the drug-conjugates by surface enhanced Raman scattering (SERS) and simulated Raman scattering (SRS). The results could reveal new mechanistic insights into the mode of action of the drug conjugates. Overall, this work illustrated the impact of nanotechnology in cancer treatment and supported the development of a robust model for the delivery of cisplatin in cancer cells.

(BIM-05.5) Multiplexed 3D Detection of Antibody-conjugated Shell-isolated Gold Nanotags Using SERS for Breast Cancer Phenotyping

Melissa Benison¹, Neil C. Shand², Duncan Graham³, Karen Faulds³; ¹*University of Strathclyde*, ²*The Defence Science and Technology Laboratory (DSTL)*, ³*The University of Strathclyde*

Ability to image and gain molecularly specific information on cancer cell biomarker distribution.

Breast cancer is the most common cancer in the UK, accounting for 15% of new cancer cases between 2016-2018, with over 70% of cases diagnosed at stage I or stage II, and negligible numbers detected at the early clinical stage. Early detection combined with identification of the cancer phenotype is crucial to enabling rapid and targeted treatment routes for each patient, ultimately leading to personalised therapy and improved survival rates. Three biomarkers found in cancer cells are used in breast cancer diagnosis: Estrogen receptor Alpha (ER α), Human Epidermal Growth Factor 2 (HER2) and the Progesterone Receptor (PR). Their expression delineates three general phenotypes: hormone positive cancers which overexpress ER α and/or PR. Estrogen and progesterone are hormones which help regulate breast cell growth and differentiation, therefore overexpression promotes uncontrolled malignant cell growth. Hormone negative phenotypes show high expression of HER2, with below average levels of ER α and PR. Triple negative cancers conversely show no heightened expression of ER α , HER2 or PR.

The ability to simultaneously detect biomarker expression by imaging cancer cells would expedite the diagnostic procedure by providing early information on cancer phenotype, enabling a more targeted therapy route to be implemented. To this end, surface-enhanced Raman spectroscopy (SERS) was used to 3D image hormone-positive MCF7 and hormone-negative SKBR3 breast cancer cells after incubation with ER α and HER2 targeting nanotags. 3D SERS imaging of the Raman reporter signal confirmed that ER α targeting nanotags accumulated in the cytoplasm of MCF7 cells, whilst HER2 nanotags remained bound to the membrane of SKBR3 cells, results consistent with immunofluorescence staining results on biomarker localisation. Principal component analysis (PCA) enabled the multiplexed detection of the two nanotags in 3D SERS imaging of the MCF7 and SKBR3 cells due to the distinct spectral signature and narrow bands characteristic of the SERS spectra of each nanotag. The selectivity and specificity of the two nanotags has also been investigated using 3D SERS imaging allowing the proposed SERS-based breast cancer phenotyping to be achieved.

22CHEM06: Pathways to Autonomous Chemometrics

Chair: John Kalivas

(CHEM-06.1) **Automatic Approaches for Efficient Curve Resolution of Spectral Imaging Data**

Cyril Ruckebusch¹, Laureen COIC, Raffaele Vitale¹, Cyril Ruckebusch¹; ¹*University of Lille*

In chemometrics, multivariate curve resolution of spectral mixtures is inherently a very difficult procedure to automatize because of the fact that the number and profiles of the individual components of the factor model underlying noisy spectral data is by definition unknown.

One example is the selection of *essential* spectral pixels [1-3]. in the context of chemical imaging experiments (such as Raman hyperspectral microscopy) that lead to the generation of massive though extremely redundant data. Recent works have highlighted the relevance and practical usefulness of this data selection for multivariate curve resolution - alternating least squares (MCR-ALS), however the identification of these points is not straightforward and practical solutions requires choices to be made by the user, such as choosing the dimensionality of the principal component analysis subspace in which to perform convex hull calculation [2], or manual tuning of control parameters to weaken the effect of the non-negativity constraint [4]. In this presentation, we will explore an alternative approach based on discrete Fourier transformation of the original data, which provides a way to extract essential information from a planar representation, whatever the dimensionality of the problem and the number of components involved. Effect of noise will be investigated and applications will cover different types of challenging data sets. On the way towards automatic multivariate curve resolution, another hurdle is related to bilinear matrix decomposition, which is not unique due to rotational ambiguity. On this side, in the context of fluorescence microscopy imaging, we will show how the properties of the measured signals can be exploited to generate three-way data arrays from the originally measured two-way data matrices, which guarantees uniqueness of the decompositions achieved. This can be very useful since the application of multilinear factor decomposition algorithms such as parallel factor analysis (PARAFAC) or multivariate curve resolution – alternating

(CHEM-06.2) **Self-Optimizing Support Vector Classifiers Applied to the Analysis of Maca Metabolomic Mass Spectral Profiles**

Peter B. Harrington¹, Peter B. Harrington¹, Qudus Ayodeji Thanni; ¹*Ohio University*

Support vector classifiers are often used in conjunction with kernels to accommodate classifications when there is no linear separation. The most popular kernel used in combination with the support vector classifier (SVC) is the radial basis function (RBF). The RBF function is optimized with a width parameter σ and the SVC with a cost parameter C . These parameters are usually optimized by a grid search of the training data that may lead to overfitting. Bootstrapped Latin partitions (BLPs) provide a comprehensive and general approach for calculating average prediction errors. The optimal parameters can then be obtained from response surface modeling (RSM) of a grid formed from the design points of (σ, C) . This self-optimizing RBF-SVC (SO-RBF-SVC) is easy to employ and compared favorably to other classifiers on a dataset comprising direct infusion negative ion high-resolution mass spectra of Maca extracts from Peru and China.

Table of external validation using 100 bootstraps and 4 Latin partitions for classifying Maca extracts by their country of origin using normalized negative ion mass spectra.

SVC-Tree

SO-PLS-DA

SO-RBF-SVC

SO-SVC

93.8±0.5%

86.5±0.9%

95.1±0.8%

93.8±0.5%

(CHEM-06.3) **Autonomous Chemometrics, Is Resistance Futile?**

John H. Kalivas¹, Jordan Peper¹, Nathan Woods, Rajiv Khadka, Xingyue Yang, John Koudelka; ¹*Idaho State University*

Multivariate calibration and classification are two key data analysis areas involving training and prediction. Regardless of the algorithm, the methods generally need to be tuned and optimized with respect to representative calibration samples and adjustable tuning parameters for accurate predictions. Numerous empirical approaches exist, but there is an ever-increasing push to automate the optimizations. It is said that by using automated processes, including artificial intelligence (AI), cognitive human expertise biases can be removed that could lead to wrong predictions. However, human biases still exists because the systems (algorithms) are built and programmed by humans. For example, a program that automatically determines similarity between two spectra is biased by the similarity measure choice. To counter this effect, multiple similarity measures can be fused but ultimately, an algorithmic fused number is still used to make automated decisions. Problems can sometimes arise when using such fused scores such as similar fused values but for different reasons (degenerate situations). Data visualization (DV) approaches have been developed to assist humans in making modeling decisions. However, many DV approaches are fairly static and limited in the number of variables characterized in a graphic. For example, even with the popular principal component analysis tool projecting high dimensional data structures to lower dimensions, the high dimensional information can be to spread out by the projections causing the information to be unrecognizable. Attempts have been made to expand the multidimensionality of graphics with Chernoff faces where variables are represented by a cartoon of face features. It was found that many essential data patterns (clusters) could be grasped and identified by an untrained human. Novel interactive dynamic data visualization tools using immersive virtual reality (VR) platforms are

maturing for visual data mining. Results are presented for various data analysis situation using multiple near infrared (NIR) spectral data sets demonstrating how a human can visually data mine with graphics and VR for discovery purposes as well as for making decisions.

(CHEM-06.4) A Digital Science Platform for Process Chemometric Model Maintenance.

David A. Joyce¹, Steve McCann¹, Kenneth Gonzalez¹, Gary Walters¹; ¹*Thermo Fisher Scientific*

Creating, monitoring and maintaining online chemometric models at scale using LIMS , SDMS & SPC.

The presentation will illustrate a practical system for the creation, monitoring and maintenance at scale of process spectroscopy chemometric models and reference data.

We will describe how a multi-plant, multi-instrument cloud installation of a software platform comprising a standard Laboratory Information Management System (LIMS) with integral Scientific Data Management System (SDMS) coupled with integration middleware was used to address some of the key challenges faced in process spectroscopy data management. Models are centrally managed, version controlled and are deployed from a central database. Model performance is monitored via Statistical Process Control (SPC) charts and any significant deviation flagged to operators. Spectra are routinely captured from the online analysers and stored in the SDMS. Reference lab results are captured from daily grab samples, compared with online results and stored in the LIMS. Should a model need to be re-built the process of creating the training data set is automated allowing rapid incorporation of new spectra and reference data. The new model may then be validated against historical spectra from the online analyser pulled by query from the SDMS. The platform reduces the scope for error and ensures that a small staff of chemometricians can manage a large and growing fleet of online analysers - with a direct and tangible benefit to the profitability of the plant. We will also discuss the use of a cloud platform to deliver this and other capabilities efficiently beyond a single plant.

(CHEM-06.5) Monitoring Worker Exposure to Respirable Crystalline Silica: Application for Data-driven Predictive Modeling for End-of-Shift Exposure Assessment

Cody Wolfe¹, Lauren Chubb¹, Rachel Walker¹, Yekich Milan¹, Emanuele Cauda¹;
¹*CDC/NIOSH*

PLS modeling using field-generated data to predict exposure to respirable crystalline silica reduces information latency.

In the ever-expanding complexities of the modern-day mining workplace, the continual monitoring of a safe and healthy work environment is a growing challenge. One specific workplace exposure concern is the inhalation of dust containing respirable crystalline silica (RCS) which can lead to silicosis, a potentially fatal lung disease. This is a recognized and regulated health hazard, commonly found in mining. The current methodologies to monitor this type of exposure involve distributed sample collection followed by costly and relatively lengthy follow-up laboratory analysis. To address this concern, we have investigated a data-driven predictive modeling pipeline to predict the amount of silica deposition quickly and accurately on a filter within minutes of sample collection completion. This field-based silica monitoring technique involves the use of small, and easily deployable, Fourier transform infrared (FTIR) spectrometers used for data collection followed by multivariate regression methodologies including Principal Component Analysis (PCA) and Partial Least Squares (PLS). Given the complex nature of respirable dust mixtures, there is an increasing need to account for multiple variables quickly and efficiently during analysis. This analysis consists of several quality control steps including data normalization, PCA and PLS outlier detection, as well as applying correction factors based on the sampler and cassette used for sample collection. While outside the scope of this article to test, these quality control steps will allow for the acceptance of data from many different FTIR instruments and sampling types, thus increasing the overall useability of this method. Additionally, any sample analyzed through the model and validated using a secondary method can be incorporated into the training dataset creating an ever-growing, more robust predictive model. Multivariate predictive modeling has far-reaching implications given its speed, cost, and scalability compared to conventional approaches. This contribution presents the application of PCA and PLS as part of a computational pipeline approach to predict the amount of a deposited mineral of interest using FTIR data. For this specific application, we have developed the model to analyze respirable crystalline silica, although this process can be implemented in the analysis of any IR-active mineral, and this pipeline applied to any FTIR data.

22IR01: NanoIR in Material Science

Chair: Georg Ramer

(IR-01.1) High Throughput Imaging of Composition, Thermal Conductivity and Interfacial Thermal Conductance with Nanoscale Resolution

Andrea Centrone¹, Mingkang Wang¹, Georg Ramer², Georges Pavlidis³, Jeffrey J. Schwartz⁴, Vladimir Aksyuk¹; ¹*National Institute of Standards and Technology*, ²*TU Wien*, ³*University of Connecticut*, ⁴*Laboratory for Physical Sciences*

Photothermal induced resonance (PTIR)[1,2], also known as AFM-IR, is a scanning probe technique that uses the tip on an AFM to transduce the sample photothermal expansion to enable IR spectroscopy at the nanoscale. However, conventional AFM probes do not have sufficient sensitivity or bandwidth to capture the fast sample thermalization linked to the sample thermal conductivity (η) and interfacial thermal conductance (G). Measuring η and G at the nanoscale is critical for engineering thermoelectrics, memristors and for studying thermal transport.

Time-domain thermoreflectance (TDTR) is a pump-probe technique that measures η and G by reconstructing the sample time-domain photothermal expansion as a function of the probe delay time. However, TDTR spatial resolution is limited to the micrometer scale and requires long measurement times (≈ 120 s per point).

Here, we develop an optomechanical cantilever probe and customize PTIR setup to directly capture, at once, the entire time-domain sample thermal expansion due to the absorption of IR laser pulses with nanoscale spatial resolution, ≈ 10 ns temporal resolution and high sensitivity concurrently, thanks to a low detection noise (≈ 1 fm/Hz^{1/2}) over a wide (>100 MHz) bandwidth. Such setup enables measuring IR spectra and nanoimaging of composition, η and G with a throughput $\approx 6000\times$ faster (20 ms per pixel) than macroscale-resolution TDTR and $\approx 500000\times$ faster than for PTIR measurements with conventional AFM cantilevers (ringdown). As a proof-of-principle demonstration, we obtain 100×100 pixel maps of η and G in 200 s with a small relative uncertainty (5%) on a ≈ 3 μm wide polymer particle and measure the IR spectra of a molecular monolayer [3]. This work paves the way to study composition along fast thermal dynamics in materials and devices with nanoscale resolution.

Finally, I will discuss why the high resonance frequency (10 MHz) of this new probe is ideal for obtaining IR spectra and images with improved spatial resolution.

[1]Centrone, A., *Annu. Rev. Anal. Chem.* **2015**, 8 (1), 101-126.

[2]Kurouski, D. ; Dazzi, A.; Zenobi, R. ; Centrone, A, *Chem. Soc. Rev.*, **2020**, 49, 3315-3347

[3]Cahe, J.; et al, *Nano Lett.* **2017**, 17, 5587–5594

(IR-01.2) **Subsurface Imaging and Spectroscopy in Two-Dimensional Materials via Photothermal Induced Resonance**

Jeffrey J. Schwartz¹, Andrea Centrone²; ¹Laboratory for Physical Sciences, ²National Institute of Standards and Technology

Due to their weakly bound, layered crystal structures, two-dimensional (2D) materials can be exfoliated as thin nanosheets down to the monolayer limit. These sheets exhibit a wide range of interesting phenomena and useful properties owing to their size as well as to interactions with their surroundings. Photothermal induced resonance (PTIR) combines capabilities of atomic force microscopy and far-field infrared (IR) absorption spectroscopy to enable imaging and spectroscopic characterization of materials with nanoscale (< 20 nm)

resolutions. Unlike some other nanoscopic techniques that are primarily sensitive to the outermost sample surface, PTIR can detect IR absorption at micron-scale depths. Here, we apply PTIR to examine subsurface IR absorption in two different 2D material systems: van der Waals heterostructures composed of stacked nanosheets trapping subsurface contaminants, and MoO₃ single-crystals that permit propagation of hyperbolic phonon polaritons when strongly coupled with mid-IR light. We identify small quantities of trapped polymers between nanosheets due to their characteristic IR absorption peaks known from far-field databases, indicating likely contamination sources and remediation strategies. Further, we image phonon polariton propagation in MoO₃ crystals to determine the influence of different substrate materials and morphologies, as well as to detect subsurface crystal defects and buried contaminants that alter the dielectric environments evanescently sensed by propagating polaritons. Subsurface imaging and spectroscopic measurements, such as these, reveal important material characteristics, including the presence of materials or defects otherwise hidden to many sensing techniques. These characteristics are especially important to the design and fabrication of precisely engineered 2D material devices and have far-reaching applications across a variety of disciplines.

(IR-01.3) A Closer Look at a Post-Consumer Recycled Polyolefin Blend: Chemical Characterization at the Nanoscale Using Tapping Mode AFM-IR

A. Catarina V.D dos Santos¹, Davide Tranchida, Bernhard Lendl¹, Georg Ramer¹; ¹*TU Wien*

The recycling of polyolefins such as polypropylene (PP) and polyethylene (PE) plays an important role in the reduction of the amount of improperly disposed plastic waste, as they are among the most common polymers produced. Sorting is an important, but tedious step of the recycling process, where the polymer waste is separated into different polymer types to avoid contamination that leads to a deterioration of the final blend's mechanical properties. Therefore, blends produced directly from heterogeneous waste streams that retain desirable properties and avoid thorough waste separation are potential cost-efficient alternatives to virgin materials. A blend's nanoscale structure and chemical composition has great impact on its properties; however, current nanoscale characterization of polymers such as TEM, SEM, and AFM rely on staining, or previous knowledge of the sample's chemical composition to obtain chemical information. These approaches may prove insufficient for the analysis of complex recyclates, where the composition is not as predictable as in virgin polymers

AFM-IR (also known as PTIR), is a scanning probe-based nanoscale IR technique that combines the resolution of an AFM with the chemical information provided by IR spectroscopy. For the analysis of polymers, tapping mode AFM-IR is preferred over contact mode, due to its better resolution and smaller sensitivity to changes in the sample's mechanical properties. In this work a PE/PP/rubber blend derived entirely from post-consumer waste collected at the municipal level is analysed using tapping mode AFM-IR. Several key features were identified, including sub-micron sized contaminant polymer particles (polyamide and polyurethane), and small (≈ 500 nm diameter) PP droplets present inside the PE phase. Most interestingly, the interface between PE and PP was identified as EPR rubber, through the application of a gaussian mixture model to the IR maps. To confirm this result, full-length AFM-IR spectra obtained in the same location were also analysed. Phase distributions derived from these spectra were found to agree with those in the IR

images. Reference TEM measurements further confirmed the information provided by AFM-IR.

We believe this work demonstrates the applicability and usefulness of AFM-IR to the routine analysis of complex polymers recycles at the nanoscale.

(IR-01.4) Nanoscale Infrared Study of Ryugu Samples Returned by the Hayabusa 2 Space Mission

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First infrared characterization at the nanoscale of asteroid particles returned from a spatial mission (Hayabusa2)

The distribution of chemical bonds in organic matter (OM) of interplanetary samples (meteorites and micrometeorites) can be efficiently and non-destructively characterized using infrared (IR) vibrational spectroscopy. Conventional IR microscopy provides a global view of the dust grain physico-chemical composition but remains spatially limited by diffraction. With synchrotron-based μ -FTIR microscopy, spot sizes of a few microns at best can be achieved in the mid-IR range. Such diffraction limited sampling can be circumvented by using AFM-IR microscopy.

This study focuses on precious samples from the Japanese space mission Hayabusa2, that returned samples from the primitive asteroid Ryugu. Ryugu samples were received from the "Insoluble Organic Matter - IOM" and "Stone" initial analysis teams led by Dr. H. Yabuta and Dr. T. Nakamura, respectively. Several samples from two different sample chambers (A and C, corresponding to two different collecting sites) were prepared by crushing small fragments on diamond windows. The analyzed areas were chosen based on previous μ -FTIR synchrotron analyses. With large scale analysis (20 μ m x 20 μ m), the main components of the sample (i.e. mineral phases) were characterized with a spatial resolution of \sim 75 nm. As the OM contribution in the IR spectra is weaker than that of minerals, a localized and highly spatially resolved analysis was made using the new IconIR system. Maps of 3x3 μ m at a spatial resolution of \sim 25 nm were then acquired. The size of the OM inclusions characterized with the IconIR system range from 50 nm to 500 nm. On the largest inclusion, the OM signal is a mix between C=C and C=O, but chemical heterogeneities are observed at small scales: parts of the inclusion seem to exhibit local enrichment in C=O while a local enrichment in C=C is also observed.

These results demonstrate the presence of organic inclusions intimately mixed with minerals in Ryugu samples at the sub-micron scale, directly on the whole rock samples. It also provides insight into the distribution of organic functionalities of the OM in whole rock that will be compared to IOM extracted from chamber A and C samples by acid treatment to remove the mineral phases.

(IR-01.5) Chemical Nano-Speciation of Breast Microcalcifications in Cancerous Tissues: the Potential of AFM-IR Technique to Decipher Microcalcification Genesis

Margaux Petay¹, Ariane Deniset-Besseau¹, Alexandre Dazzi¹, Maguy Cherfan², Dominique Bazin¹; ¹University Paris-Saclay/CNRS, ²GHT NOVO

Nano-investigation of microcalcifications in breast tissue to decipher their possible pathological role.

Infrared (IR) spectromicroscopy is a valuable tool in medicine for the chemical speciation of biological samples. However, its spatial resolution is close to a few microns in the mid-IR spectral region, which can be challenging for the understanding of pathological mechanisms at the nanometric level. In the breast, calcium-based deposits, also called breast microcalcifications (BMCs) are considered benchmarks of breast cancer. It has been emphasized that BMCs' chemical composition might be correlated to cancer and its malignancy [1,2]. Yet, mineralization processes in the breast are not well understood, nor is the link between the chemical speciation of BMC and the pathology stage. Our project aims to provide an insightful description of BMCs' chemistry to better understand their relationship with cancer. In that regard, we established a multiscale approach for the morphological and physical-chemical characterization of BMCs inside human breast biopsies. Especially, using AFM-IR, an infrared nanospectroscopy technique [3], we successfully characterized at the nanometer scale micrometric and nanometric BMCs in their native environment. Without specific sample preparation than those implemented in hospitals, we were able to reveal chemical heterogeneities at the nanometric level. Currently, two types of BMC are distinguished based on their chemistry: type I made of calcium oxalate dihydrate and type II made of calcium phosphate apatite; but our results suggest that BMCs might be composed of, not one, but a mix of these two types, as well as two other chemical phases: amorphous carbonated calcium phosphate and whitlockite. These findings emphasize the potential of AFM-IR to depict mineralization in the breast and to provide insights into BMC chemistry necessary to understand the correlation between BMCs and the pathology. [1] Baker et al., New relationships between breast microcalcifications and cancer, *Br. J. Cancer*, 2010. [2]

Scott et al., N. Stone, Relationships between pathology and crystal structure in breast calcifications: An in-situ X-ray diffraction study in histological sections, *Npj Breast Cancer*, 2016. [3] Mathurin et al., Photothermal AFM-IR spectroscopy and imaging: Status, challenges, and trends, *J. Appl. Phys.*, 2022.

22LIBS06: Space Applications

Chair: Pablo Sobron

(LIBS-06.1) Exploring the Lunar Surface and Volatiles with Laser-Induced Breakdown Spectroscopy

Jeffrey Gillis-Davis¹, Jeffrey Gillis-Davis¹, Pablo Sobron², Bradley Jolliff; ¹*Washington University in St. Louis*, ²*Impossible Sensing*

A new wave of lunar missions is upon us. They seek to explore geologically diverse field sites, discover previously unsampled rock types, and characterize volatile-rich polar regions. In-situ sample investigations—coupled with remote sensing data—are required to truly understand the process(es) that form materials that now exist on the surface of the Moon. Laser-induced breakdown spectroscopy (LIBS) is an active analytical technique that can classify lunar rock types and assay volatile resources. Compositional data for rocks and soils is paramount for assessing the diversity of lunar rock types: It informs lunar science goals of how the Moon differentiated, the nature of its interior, and the stratigraphy of the crust. LIBS-based major (e.g., Si, Al, Ca, Fe, Ti, Mg, Na, and K) and trace elements (e.g., P, Ba, Th) abundances can effectively discriminate between different lunar rock types: e.g., feldspathic highlands primary crust, magmatic magnesian suite (Mg-suite), mare basalts of various compositions (VLT, low-Ti, High-Ti, High-K, etc.), and rocks with elevated Potassium, Rare Earth Element, and Phosphorus-rich (KREEP-rich).

Another goal of renewed lunar exploration focuses on furthering our knowledge of the abundance, distribution, composition, and origin of the Moon's near-surface volatiles. Understanding the nature of the Moon's polar volatiles could provide insight into the origin, the timing of delivery, and subsequent evolution of water and volatiles in the inner Solar System. Further, water on the Moon is of great interest as a future resource for astronauts and robotic missions. LIBS measurements of ice deposits would provide an opportunity to test hypotheses regarding the delivery and retention of water and other volatiles in the inner Solar System. LIBS could also measure minute quantities of purported organics, fluid inclusions, and individual dust particles within the ice. Repetitive laser pulses could ablate away ice layers to bore through the layered materials and obtain a record of the elemental composition of each ice layer or to examine subsurface soil deposits. Hence, LIBS measurements would be able to answer major outstanding questions regarding the lunar rock element compositions, illuminate the origins of lunar water, and evaluate volatile ices for the potential of in-situ resource utilization.

(LIBS-06.2) LIBS, Raman, and Chemometrics for Exploration of Ocean Worlds

Laura E. Rodriguez¹, Laura E. Rodriguez¹, Anastasia Yanchilina², Kirby Simon², Evan Eshelman², Deborah Kelley³, Pablo Sobron², Laurie Barge¹; ¹NASA Jet Propulsion Laboratory, California Institute of Technology, ²Impossible Sensing, ³University of Washington

Ocean worlds such as Europa and Enceladus are prime targets in the search for extraterrestrial life as they have an abundance of liquid water and are likely host to geochemical gradients which can serve as an energy source to sustain life. Importantly, strong geochemical gradients are most likely to form wherever hot fluids interact with rocky crust—conditions that also favor the formation of hydrothermal vents. Hydrothermal vents are hotspots of geochemical and biological activity in Earth's oceans and may generate conditions that facilitate abiogenesis on a prebiotic world. Given this, it is imperative to test the feasibility of mission ready techniques for characterizing hydrothermal vent precipitates. Raman and Laser Induced Breakdown Spectroscopy (LIBS) are of particular interest given that they are complimentary and can provide insight into the habitability of ocean worlds: LIBS can detect all of the biogenic elements needed for life (CHONPS) whereas Raman provides information on mineralogy and general organic trends.

We investigated the utility of several pre-processing techniques (denoising, peak detection, etc.) and machine learning strategies (e.g. Partial Least Squares (PLS), Multivariate Curve Resolution (MCR), Hierarchical clustering, data fusion) to discern elemental, mineralogical, and organic trends within the samples. Subsamples were analyzed on a pay per sample basis to ground truth results, namely total carbon, total organic carbon, elemental abundances (via Inductively coupled plasma atomic emission spectroscopy) and mineralogy (via quantitative X-ray diffraction).

We generated high resolution Raman and LIBS maps from the analysis of hydrothermal vent precipitates. Preliminary analysis has shown that LIBS alone is sufficient for discerning elemental and mineralogical trends (when used as input for MCR) within hydrothermal vent precipitates. Mineralogy deduced by the MCR analysis of Raman data were also in agreement with both LIBS and XRD results. Regarding organic carbon, we found that data fusion of Raman and LIBS significantly improved the ability for a PLS model to predict the abundance of organic materials. Given the mission heritage of these instruments (Raman and LIBS are on NASA's *Perseverance* Mars rover; LIBS is also on NASA's *Curiosity* rover), these techniques are promising tools for future ocean world exploration.

(LIBS-06.3) **LIBS for Exploring the Clouds of Venus**

Kirby Simon¹, Pablo Sobron¹, Anastasia Yanchilina¹, Diana Gentry², Laura Iraci², Alfonso Davila², Andrew Mattioda², Amanda Brecht², Alan Cassell²; ¹Impossible Sensing, ²NASA Ames Research Center

With NASA's VERITAS and DAVINCI missions and ESA's EnVision due to launch in the upcoming decade, there will soon be a plethora of new data describing the physical and chemical properties of Venus's atmosphere and surface. Despite the growing interest in Venus scientific exploration however, there is insufficient in-situ data to answer key

questions around Venus's cloud chemistry and composition, needed to address knowledge gaps such as: What is the nature and identity of the unknown ultraviolet (UV) absorber? How are sulfur species cycled? How much water is contained within Venus's aerosols? Laser Induced Breakdown Spectroscopy (LIBS) is a powerful technique that could provide in-situ, high resolution data related to the elemental composition of gases, particles, and liquid droplets present in the atmosphere and cloud layers of Venus. LIBS is fast (kHz), sensitive (ppm to ppb), requires no sample processing nor manipulation prior to measurement, and can be designed into a small footprint (size, weight, and power) instrument, making it an ideal technique for characterizing Venus's atmosphere on a descent probe or aerial platform. A LIBS instrument could provide a complete elemental profile, in real-time, of atmospheric samples collected during a transect of the clouds.

LIBS instrumentation can also seamlessly integrate additional spectroscopic measurement techniques such as Raman spectroscopy and ultraviolet fluorescence spectroscopy into a single optomechanical assembly. Combining complementary spectroscopic techniques with LIBS enables data fusion of measurements that further enhances the scientific output of the instrument without significantly increasing the instrument footprint nor impacting mission complexity.

In this work, we investigated the elemental composition of Venus atmospheric analog samples using in-house LIBS instrumentation developed for planetary science applications. Even with chemically and physically complex samples, with a single LIBS measurement we can detect and identify both major and trace elements. We performed additional spectroscopic measurements on these samples and explored the data fusion between LIBS and these measurement sets to better understand the gaps in spectroscopic data and optimize the scientific output of the instrument. Finally, we developed several notional instrument concepts that could be used to characterize Venus's atmosphere and cloud chemistry in-situ in different mission architectures.

(LIBS-06.4) Using Laboratory LIBS Acoustics Experiments to Elucidate SuperCam Microphone Data on Mars.

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In February 2021, we were performing a series of laser-induced breakdown spectroscopy (LIBS)

combined with acoustic experiments in a large (~2m long by 1 m diameter cylinder) thermalvacuum

(TVAC) chamber at the Los Alamos National Laboratory (LANL). LIBS works by focusing laser energy at the surface of a sample, creating a plasma whose optical spectrum reveals the elemental composition of the sample. During the first μ s of the plasma expansion, when performed under an atmosphere, a shock wave is created and subsequently relaxes into an acoustic wave. Recordings this laser-induced acoustic signal is complementary to LIBS analysis

as the shock wave generation depends on material physical properties such as hardness, optical

absorption.... However, acoustic wave dynamics strongly depend on atmosphere conditions.

Laboratory experiments in a controlled atmosphere are needed to interpret data coming from Mars.

Several rock samples were placed at one end of the TVAC chamber. By firing through the chamber window, a BigSky/Quantel class 4 laser, operating at 20 Hz, with energies ranging from 11 to 59 mJ/pulse, produced a series of LIBS sparks (250 to 350 shots per location) on the

samples. Two high-sensitivity Brüel Kjør (B&K) microphones, operating in the 20Hz to 20kHz

frequency range, were placed at 28cm and 67cm from the sample plate. For each shot, digitization at 1MHz of the acoustic signals was triggered by the laser pulse. This enabled a measurement of the arrival time of the acoustic signal produced by LIBS with high-precision.

We analyze in particular, a series of LIBS shots performed on a sandstone sample. Our measurements reveal that the acoustic arrival time is dependent on the sound peak pressure showing time delays of about 0.02ms for change of peak pressure of 10Pa. The latter are related to material heterogeneity and the formation of a crater. This behavior was measured for different temperature and pressure conditions and is characteristic of non-linear processes

inherited from the shockwave generation. A theoretical model is also developed to quantify the

extent of the non-linear regime and to verify that the observed variations of propagation time

observed are indeed due to non-linearity.

(LIBS-06.5) New Insights in Autonomous LIBS-based Planetary Exploration: Generalized Scale Invariant Quantitative LIBS

Pablo Sobron¹, Daniel Van Hoesen; *Impossible Sensing*

Technological advancements in the past two decades have unlocked the ability of LIBS to record spectral data in real time. However, analytics—turning real-time data into real-time compositional knowledge—has lagged behind: only recently Anderson et al. (2022), building on data from Perseverance’s SuperCam LIBS instrument, have demonstrated that elemental abundances of unknown targets can be predicted within a few weight percent. This is promising because the regression algorithms used by Anderson et al. and new machine learning tools can effectively bring real-time autonomy and quantification to LIBS applications. The challenge here is that, to date, each model developed for quantitative LIBS has been tailored to a specific instrument and it doesn’t translate to others. This limits both the reproducibility and the ability to share models between instruments. Ultimately, this is a barrier to generalized adoption of LIBS as an off-the-shelf tool for planetary exploration applications such as resource prospecting on the Moon, where autonomous rovers will likely mount different types of LIBS systems, each optimized for specific exploration tasks. How can we turn LIBS into a technique for generalized quantitative analysis?

Convolutional Neural Networks (CNNs) are robust methods that learn scale invariance through pooling where the data size is reduced to learn scale parameters from the instrument or environment. This is most notably seen in autonomous vehicles where cameras must identify objects at various scales. In LIBS, detector specifications, temperature, pressure, laser wavelength and power, and measurement distance markedly affect spectra. CNNs can learn environmental properties providing confidence that generalized prediction models can be created for different instruments deployed in various environments. In practice, this means that CNNs add adaptability to LIBS and enable intelligent exploration of unknown environments by autonomous robots. Our team is developing base prediction models for quantitative LIBS that use initial training on data from several instruments. In this talk we show a use case where we deploy a well performing base model to a different LIBS instrument where targeted retraining is performed on a drastically smaller set of samples. This is our base for future developments geared toward bringing intelligence to planetary LIBS tools.

22MASS04: Rapid Screening and Assay Methods for Mass Spec and Beyond

Chair: Abraham Badu-Tawiah

(MASS-04.1) Ultrasensitive Detection And Quantification Of HIV DNA And Its Polymerase Chain Reaction Products By A Novel Enzyme Linked Mass Spectrometric Assay

Nan Cheng¹, Saaimatul Huq¹, Ming Miao², John G. Marshall¹; ¹*Toronto Metropolitan University*, ²*YYZ Pharmatech Inc.*

A novel and ultrasensitive method to detect nucleic acids from genetic samples and PCR

There is an urgent need to make absolute quantification of nucleic acids against external standards in clinical diagnostics, gene therapy, food or agriculture and basic biomedical research. Especially now it is widely recognized that accurate and sensitive detection of nucleic acids is critical in early diagnosis of infectious and hereditary diseases, particularly for virus pandemic prevention. The quantification of an enzyme linked DNA hybridization assay by mass spectrometry of the small molecule products yielded an ultrasensitive technology for nucleic acid detection, DNA enzyme linked mass spectrometric assay (DNA ELiMSA). It combines the powerful amplification enzyme alkaline phosphatase conjugated with streptavidin (APSA) that binds biotinylated DNA detection probes with high affinity for analysis by mass spectrometry. Polymerase chain reactions (PCRs) that are below detection by traditional fluorescence methods, or genetic samples without PCR amplification, may be sensitively and specifically quantified by ELiMSA. Here we applied this technique to detect HIV PCR products and HIV plasmid spiked into serum without PCR amplification. A synthetic HIV DNA oligonucleotide was used to determine the quantitative concentration range of HIV DNA molecules. HIV PCR products were then

analyzed by the agarose gel electrophoresis and the DNA ELiMSA respectively. It was found that the ELiMSA was 10 to 100 times more sensitive than the traditional fluorescence detection. The application of DNA ELiMSA to HIV plasmid samples spiked into fetal bovine serum reached about 10 pM to 50 nM HIV plasmid with good linearity without prior DNA extraction. Thus, HIV DNA ELiMSA combined DNA hybridization and enzyme amplification to analyze the small molecule reporter adenosine with a linear and sensitive detection by mass spectrometry that quantified PCRs far beyond typical fluorescence methods, and provided amplification-free detection of HIV DNA directly from serum even without extraction.

(MASS-04.2) Rapid Screening of Suspect Drug Products Containing Designer Benzodiazepines Using DART-MS

Skyler W. Smith¹, Travis M. Falconer¹, Sara E. Kern², John P. Roetting¹, Martin K. Kimani¹; ¹*U.S. Food & Drug Administration*, ²*US Food and Drug Administration*

Comparing detection capabilities of rapid screening MS techniques for designer benzodiazepines in suspect drug products

Counterfeit or illicit drug products are an important public health concern in the U.S. as deaths have continued to rise over the past two decades due to drug overdoses. The Forensic Chemistry Center (FCC) of the U.S. Food & Drug Administration (FDA) routinely provides the FDA Office of Criminal Investigations with laboratory-based services to investigate counterfeit or illicit drug products suspected to contain approved and/or unapproved drugs, especially tablets and powders. FCC regularly receives a considerable number of these tablets and powders containing undeclared active pharmaceutical ingredients (API) or drugs that can endanger public health. Additionally, these products containing undeclared APIs and/or unapproved drugs could be encountered at international mail facilities and express courier hubs and enter the U.S. supply chain. Therefore, the development of rapid screening techniques that can effectively detect violative products is an area of interest to the FDA and at the FCC.

Designer benzodiazepines, that are not approved by the U.S. FDA, have been commonly identified in recent suspect sample submissions. Direct analysis in real time ambient ionization mass spectrometry (DART-MS) has emerged as a powerful tool in rapid screening. The DART ionization source has the ability to be coupled to a portable mass spectrometer for use outside the laboratory or a high resolution instrument within the laboratory. In this work, various suspect tablets and powders containing one or more designer benzodiazepines were analyzed using an IonSense DART ionization source

coupled to a Waters QDa detector with a thermal desorption unit or Thermo Scientific Q Exactive Orbitrap. Using these instruments, eight designer benzodiazepines (clonazepam, diclazepam, etizolam, flualprazolam, flubromazepam, flubromazolam, meclonazepam, and metizolam) were among the drugs detected in the various suspect samples. Following rapid screening, the analyte(s) of interest in each sample were confirmed and quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a SCIEX QTRAP 6500+ system. The advantages and disadvantages of the two rapid screening configurations and the confirmation technique will be discussed, including sample preparation considerations, instrumental capabilities (such as accuracy, precision, minimal detectable level, multi-component detection), identification requirements, and data collection and analysis.

(MASS-04.3) Improvements and Characterization of Localized-Sustained Stimulation of Brain Slices On-Chip

Colby E. Witt¹, Ashley E. Ross¹; ¹*University of Cincinnati*

First, sustained and localized microfluidic platform to study ischemic stroke events

Strokes (ischemic and hemorrhagic) accounts for 5th leading cause of death in USA placing this as a large global burden. Previous work has gone into understanding the behavior of neurochemicals during global ischemic events. However, there is a large shift within the neuroscience community to study these traumatic events at the site of the injury. By coupling microfluidics with fast-scan cyclic voltammetry (FSCV), our lab has been able to make great strides toward making measurements at the localized sight of ischemia. Our previous work has shown that we are able to fabricate a 200 μm port on a microfluidic platform to deliver injury with a $583.5 \pm 65.9 \mu\text{m}$ spatial resolution. Furthermore, we tested the viability of our device by monitoring tissue functionality on-chip for dopaminergic release with FSCV. Though this initial work shows promise, we want to optimize the fixed delivery limited from spread and as finite as possible. Therefore, in this talk improvements to our original design as well as further neurochemical analysis of localized events will be highlighted. Ultimately, this new device enables sustained, spatially resolved delivery of injury in *ex vivo* tissue to measure directly at the sight of the stimulation in real-time. By doing so, we set our device at the precipice to bridge this gap in the literature.

(MASS-04.4) Development of Electrochemical Aptamer-Based Neuropeptide Y Sensor

Jordan M. Seibold¹, Ryan White¹, Ashley E. Ross¹; ¹*University of Cincinnati*

This sensor will allow for the detection and study of Neuropeptide Y in live tissue

Many conditions such as stress are commonly known to weaken the immune system which increases the likelihood of infections, autoimmunity, and other immunocompromised conditions.

Neuropeptide Y(NPY) is a neuropeptide that is co-packaged in sympathetic neurons in the lymph node and is involved in a multitude of important functions, including serving as an immunomodulator, regulating T cell function, and energy and hunger regulation. The molecular mechanisms that are responsible for these interactions are not well understood. As of now, there is not a way to measure sympathetic direct and localized release of NPY in tissue.

Here, we have overcome this technological challenge by developing an electrochemical aptamer-based sensor for NPY detection. Aptamers are useful biological sensors for monitoring a specific molecule because of their high selectivity and binding affinity for the target of choice. A comprehensive comparison of selected aptamers specific for NPY was done to isolate the optimal aptamer sequence for electrochemical detection. The sensor was fabricated by modifying the surface of a gold macro disk electrode with 200 nM of aptamer, followed by passivating with a self-assembled monolayer of 6-mercatohexanol. Square wave voltammetry was used to characterize the response of the sensor. Frequencies ranging from 5-800 Hz were tested and peak current was measured to determine the optimal testing frequency. The stability of detection, sensitivity, limit of detection, and specificity was determined at the optimal testing frequency. As a proof-of-principal, the sensors were used to measure known concentrations of NPY in serum at biological temperature. Overall, this provides an initial sensor framework for detecting NPY levels electrochemically with the goal of eventually enabling in-tissue detection.

(MASS-04.5) Feasibility Studies on the Cyto R1 Platform for Tumor-Associated Cell Sorting

Alexandra Hyler¹, Katherine Degen¹, Ridi Barua¹, Dean Thomas¹, Rafael Davalos², Eva Schmelz²; ¹*CytoRecovery*, ²*Virginia Tech*

The CytoR1 provides label free cell sorting and viability enrichment for downstream biomedical research applications.

The diverse cell populations that make up a tumor create a complex ecosystem that must be navigated in order to understand disease progression and develop effective treatments. Advances in sequencing and proteomic techniques have allowed an unprecedented level of detail to be collected on these heterogeneous cell populations. However, a key requirement of these techniques is a clean, highly viable sample that reflects the populations present in the tumor. If too many dead cells are present, background noise makes detecting signals difficult. Similarly, if the population heterogeneity is significantly altered, incorrect conclusions can be reached at the single-cell level. While imperfect, preparation of these samples is commonly accomplished via bead-based cell sorting techniques. This work demonstrates a novel platform's ability to generate highly viable samples with improved maintenance of heterogeneous cell populations. The Cyto R1 Platform utilizes plug and play microfluidic chips to enrich and sort cells via contactless dielectrophoresis. In direct comparisons, bead columns altered the relative composition of cell subpopulations by as much as 20%, whereas the Cyto R1 Platform never exceeded 8% deviations. The MACS column based clean ups also resulted in 50-80% sample loss depending on cell type, sample size, and starting sample condition. In contrast, the Cyto R1 returned more than 60% of sample regardless of cell types and small sample size, achieving greater than 80% recovery

in many cases. In addition to providing viability enrichment with conserved population heterogeneity, the Cyto R1 can be further used to enrich or deplete various subpopulations of interest for further refinement in downstream assays and culture. Characterization of the electro-physiological properties of various cell types allows for label-free enrichment for selected subpopulations. In one example, the Cyto R1 achieved 40% enrichment of cancer stem cells from a peripheral blood mononuclear cell mixture within 15 minutes. Similarly, fibroblasts, lymphoblasts, monocytes and cancer stem cells were found to have unique bioelectrical fingerprints that can be exploited for enrichment from mixed samples. Together, these capabilities of the Cyto R1 platform represent its novelty for sample preparation and sample enrichments for use in cancer biology, immunology, genomics, and proteomics.

22PAT01: SAS PAT Technical Section: PAT in Pharma

Chair: Jim Rydzak

(PAT-01.1) The Use of a Bench Top Simulator for Economic PAT Application Development

Stephen Hammond¹, Philip Doherty; ¹*Expo Pharma Engineering Services*

Over the last ten years, the path to effective development of PAT has evolved, driven by the advent of continuous manufacturing. The development and deployment of PAT used for pharmaceutical manufacturing has arrived at a point where application development has been miniaturized and streamlined. The availability of fast scanning instruments using fiber optic probes, integrated into processing equipment is now a standard approach for continuous systems, and the same approach can be used for traditional batch manufacturing. The development of devices that can simulate the environment of a fiber optic probe in production equipment, using a small amount of material, in a laboratory environment has enabled the development of working models for the spectrometer using the minimum amount of material, separate from the large-scale manufacturing equipment. This capability has reduced the complexity and cost of developing PAT applications for a production environment. This presentation will describe the use of benchtop simulators to streamline PAT development.

(PAT-01.2) Reactions and Crystal Form Transformations Revealed Using In-Situ Raman Spectroscopy and Optical Microscopy

Charlie Goss¹, Anthony Nocket¹, Andrew DiPietro¹, Kevin Chu¹, Swetha Ainampudi¹, Yasser Jangjou¹, Alexis Venere¹, Alicia Potuck¹, Anjan Pandey²; ¹*GlaxoSmithKline*, ²*Mettler Toledo*

This presentation will illustrate how in-situ analysis techniques like Raman spectroscopy and optical microscopy (PVM, particle vision and measurement) can be used to visualize reactions, crystallization profiles, and crystal form transformations to determine process profiles, endpoints, stability and sometimes reveal unexpected behavior. Representative Drug Substance and Drug Product example data will be presented and discussed to illustrate how these tools have helped project teams improve their process understanding and accelerate development.

(PAT-01.3) Impurity, isomer, and chiral analysis in process applications using molecular rotational resonance spectroscopy

Justin L. Neill¹, Reilly E. Sonstrom¹, Alex Mikhonin¹; ¹*BrightSpec, Inc.*

Molecular rotational resonance (MRR) spectroscopy offers a highly selective spectroscopic approach to molecular identification and quantification in mixtures. Every chemical compound has a completely distinctive MRR spectral signature that is independent of sample matrix, and directly related to the molecule's three-dimensional structure. Additionally, MRR is able to resolve all forms of chemical isomers, including regioisomers, diastereomers, isotopically labeled variants, and enantiomers. This capability enables rapid, automated, separation-free isomer analysis using MRR in process and QA/QC applications. Because MRR components can be identified directly from their three-dimensional structures, pure reference standards are not needed for each component of interest. We will present recent studies for MRR in process monitoring applications in the pharmaceutical and chemical industry.

(PAT-01.4) Improving Your Reaction Efficiency with PAT Focused Technology

Norman A. Wright¹, Brian Wittkamp¹, Charlie Rabinowitz¹; ¹*Mettler Toledo*

The growth of Process Analytical Technology (PAT) for real-time reaction analysis is clearly linked to the number of successful chemical applications that have been developed. These applications cover a broad range of academic and industrial syntheses in pharmaceutical, biopharma, chemical and polymer sciences. With success, comes an ever broadening scope and complexity of chemistries to investigate that can require increasingly innovative reaction analysis technologies to help describe both the reaction sequence and intermediates that occur during the process. The availability of infrared and Raman spectroscopies along with particle characterization provides tools to meet the challenge of many chemistry investigations by providing a higher level of reaction and process understanding. On top of these developments are increasing regulatory requirements, as well as the drive to reduce time and lower cost while achieving high quality results, all driving the expectation of higher efficiency with every experiment.

This paper will present examples of the use of synergistic PAT technologies for real-time reaction analysis. The key result from these studies is the ability to extract and combine information that can reveal information about complex chemical systems. As always, the ability to perform these analyses in real-time enables the identification, importantly of the control parameters required for optimizing a reaction without compromising product quality. These PAT methods and analyses are critical to use when optimizing and scaling up processes to ensure product yield and purity.

(PAT-01.5) Unlocking product composition using solid state Raman and Core models: From fuels to fermentation

Brian Marquardt¹, Thomas Dearing², John Richmond²; ¹*MarqMetrix*, ²*MarqMetrix, Inc.*

Raman spectroscopy is an ideal candidate for refined fuels analysis, having the key attributes of specificity, sensitivity, speed and stability and simplicity. In this paper, we will

describe the methodology for collecting high quality Raman spectra and the strategy for turning the spectral data into information to inform the decision-making process. Analysis of multiple fuel types including gasoline, jet and diesel as well as component streams such as reformate, isomerate and alkylate will be described as well as modeling strategies to ensure a simplified approach for laboratory workflow for technicians and operators

22RAM08: Raman Imaging and Microscopy

Chair: Katsumasa Fujita

(RAM-08.1) Super Resolution DO-SRS Multiplex Metabolic Imaging for Studying Aging and Diseases

Lingyan Shi¹, Lingyan Shi¹, Yajuan Li, Wenxu Zhang, Anthony Fung, Hongje Jang; ¹*UC San Diego*

Understanding the dynamics of metabolism in a multicellular organism is essential to unraveling the mechanistic basis of many biological processes in healthy and diseased conditions. There has been an urgent need of high spatial resolution, non-invasive imaging techniques for imaging metabolism of various biomolecules in cells and tissues. Deuterium oxide probed Stimulated Raman scattering (DO-SRS) can generate chemical specific metabolic imaging with high resolution, deep penetration of depth, multiplex, chemical selectivity, 3D volumetric and quantitative capability. In the present work, we developed a new approach that combines super resolution A-SUPPOSE enhanced **DO-SRS** imaging and custom designed clustering methods to visualize multiplex metabolic activities and subcellular distribution of newly synthesized macromolecules in living organisms. Within the broad vibrational spectra, we can image more than 30 different molecules including lipids subtypes-, protein-, and DNA-specific Raman profiles and develop hyperspectral detection methods to obtain multiplex imaging of various biomolecules. This technology platform is non-invasive, universal applicable, and it can be adapted into a broad range of biological studies such as neurodegeneration, aging, homeostasis, tumor progression, etc. We applied this method to study the diet regulated metabolic dynamics in animals during aging processes, the quantitative lipid and protein turnover rate, the intra-cellular metabolic heterogeneity.

(RAM-08.2) Plasmon-Enhanced Raman Nanoscopy for Probing Single Molecule Chemical Reactions

Taka-aki Yano¹; ¹*Tokushima University*

Tip-enhanced Raman scattering (TERS) microscopy has been regarded as a promising application of plasmonics for nano-imaging and nano-analysis with a nanoscale spatial resolution far beyond the diffraction limit of light. A sharp metallic probe tip plays important roles in plasmonically enhancing Raman scattering from sample molecules in the vicinity of tip. However, TERS microscopy has been still limited to passive measurement of intrinsic molecular properties and functions. Here, we proposed to utilize the plasmonic tip to locally apply various kinds of external stimuli such as pressure and voltage to sample molecules through the tip apex, enabling us to induce chemical reactions at single molecular

scale. The dynamic change in physicochemical properties can be spectroscopically probed by detecting tip-enhanced Raman scattering in response to the external stimuli.

(RAM-08.3) Application of Raman Spectroscopy to Investigate Ageing and Disease in Archaeological Human Bone

Sheona I. Shankland¹, Hugh Willmott², Alexzandra Hildred³, Adam M. Taylor¹, Jemma G. Kerns¹; ¹*Lancaster University*, ²*University of Sheffield*, ³*The Mary Rose Trust*

Access to human material for clinical research can be a limiting factor of scientific studies. Skeletal investigations can be performed on living patients using imaging techniques, which are usually X-ray based, but these only provide macroscopic information. In depth chemical analyses could advance our understanding of the skeleton; exploring changes in bone chemistry with ageing and disease. Raman spectroscopy could facilitate this without damaging samples and provide a comprehensive overview of the bone chemistry of ageing.

Archaeological remains are vital to our understanding of human history, particularly health and disease, but can also contribute to our understanding of contemporary diseases. With access to bone samples from living patients being more complex, this study aimed to use archaeological bone samples to investigate if chemical differences in the human skeleton with age and disease can be detected using Raman spectroscopy.

Bones from The Thornton Abbey Project (a medieval infirmary burial site in Lincolnshire, UK) and bones from the crew of The Mary Rose (a Tudor flagship of King Henry VIII which sank off the southern coast of England in 1545) were procured for this project. The bones from the crew of the Mary Rose are particularly well preserved due to half the ship being buried in an anaerobic seabed. These remains were separated into age categories and analysed using a 785nm Raman micro-spectrometer (Renishaw plc.). Points for analysis were selected for their biomechanical significance or to obtain the most comprehensive overall analysis of the sample.

Bones are composed of two main constituents: proteins and minerals, and results so far indicate that there are measurable chemical changes in these components associated with age. There is clear evidence that age related changes are occurring in the mineral aspect of the bones. Minerals are crucial to skeletal function as under-mineralisation results in bones that are softer than is optimal, meaning they can distort from their prescribed shape, affecting their function. Detecting these changes demonstrates that archaeological bone can successfully be used to contribute to the evolution of modern medical research.

(RAM-08.4) Opto-Lipidomics of Tissues

Mads S. Bergholt¹; ¹*King's College London*

We demonstrate the first integrated Raman and mass spectrometry instrument paving for (optical) lipidomics

Here we drive forward a new paradigm of Raman technology that enables optical lipidomic analysis of tissues. Raman spectroscopy is a label-free optical technique that can provide a point-wise vibrational molecular fingerprint of tissue “optical biopsy” for tissue diagnosis. State-of-the-art Raman spectroscopy, however, does not offer specific compositional analysis or insights into molecular biology of tissue hindering widespread adoption. This is because the vibrational Raman bands are overlapping and cannot be deciphered into the myriad of biomolecules in complex tissue. We introduce a new instrument and methodology to enable Raman lipidomic analysis. To this end, we developed a novel integrated Raman and mass spectrometry imaging system for pixel-wise correlation. We demonstrate that multivariate regression can be used to translate from the vibrational structural domain (Raman spectroscopy) into the more specific compositional lipidomic domain (mass spectrometry) thereby enabling optical lipidomics.

(RAM-08.5) Toward Photoswitchable Electronic Pre-Resonance Stimulated Raman Probes

Dongkwan Lee¹, Chenxi Qian¹, Haomin Wang¹, Lu Wei¹; ¹*Caltech*

We explored different mechanisms to develop photoswitchable raman probes.

Stimulated Raman scattering (SRS) imaging is a powerful live-cell imaging strategy with high chemical specificity and sensitivity. By utilizing pre-resonance enhanced narrow-band Raman probes, electronic pre-resonance SRS (epr-SRS) has further increased the sensitivity down to 250 nM and multiplexity up to 24 colors, enabling super-multiplex imaging. Here, we present our recent progress toward developing photoswitchable epr-SRS probes by using light-induced transitions from one electronic state to another as a strategy to switch epr-SRS signal on and off. We demonstrate that epr-SRS signals can be effectively switched off in red-absorbing organic molecules by inducing transitions to the electronic excited state, triplet state, and reduced state. We also show that photoswitchable proteins with near-infrared photoswitchable absorbance can function as the photoswitchable epr-SRS probes with desirable sensitivity (< 1 μ M) and low photofatigue (>40 cycles).

22RAM09: Spatially Offset Raman Spectroscopy

Chair: Pavel Matousek

(RAM-09.1) Design of SORS Systems for Biomedical and Art Conservation Applications

Pietro Strobbia¹, Claudia Conti², Ren A. Odion³, Tuan Vo-Dinh⁴, Pavel Matousek⁵, Marco Realini²; ¹*University of Cincinnati*, ²*Institute of Heritage Science*, ³*Duke University*, ⁴*Duke University School of Medicine*, ⁵*STFC Rutherford Appleton Laboratory*

Spatially offset Raman spectroscopy (SORS) can rely critical information in many applications, including non-invasive diagnostics and conservation science. However, each application has a different set of requirements to consider (e.g., sensitivity, depth, deployability, background signal). SORS instrumentation has to be adapted to fit the specific application and work efficiently. In this talk, we will discuss two very different applications and the relative SORS designs that were conceived. We will use these examples to talk about design rules and options in SORS. In design #1, our goal was to perform surface-enhanced SORS through the skull of a monkey (5 – 10 mm), to demonstrate the feasibility of detecting theranostics probes for brain tumors without removing the cranium. This design maximizes sensitivity and reduces footprint using inverse SORS (i.e., a variant of the basic optical SORS design). It is possible to use this variant in this design because resolving depth information is not important for this application. In design #2, our goal was to perform microSORS for art conservation. This application requires the system to be portable and to obtain depth information with high depth resolution. We achieved these requirements by designing a system using a linear fiber bundle to conserve the offset information on the detector.

(RAM-09.2) Through Bottle Quantification of Adulterated Extra Virgin Olive Oil using SORS

Royston Goodacre¹, Mehrvash Varnasseri, Yun Xu¹, Howbeer Muhamadali¹, Pavel Matousek²; ¹*The University of Liverpool*, ²*STFC Rutherford Appleton Laboratory*

It's not a question of if food fraud will occur, it's unfortunately a case of when and how badly the food supply chain will be disrupted, and whether this disruption is deliberate or accidental and if this is potentially dangerous to human and animal health. Major food adulteration incidents occur with alarming frequency and are episodic, with multiple incidents reinforcing this view. Thus, capable guardians are needed to 'police' the food supply chain.

In this study we highlight developments in through container analysis using Raman and SORS for detecting and quantifying the level of adulteration in extra virgin olive oil (EVOO) through commercially available glass bottles that are used to sell EVOO. Three types of adulterant were used including sunflower oil, pomace olive oil, or refined olive oil which were chosen due to their varying levels of chemical similarity to EVOO. We conclude that SORS has significant potential as a rapid and accurate analytical method for the non-destructive detection of adulteration of EVOO.

(RAM-09.3) Spatially Offset Raman Spectroscopy for Non-invasive Hydration Monitoring

Anna S. Rourke¹, Laura J. Elstun¹, Trevor Voss¹, Anita Mahadevan-Jansen¹; ¹*Vanderbilt University*

This work demonstrates the first utilization of spatially offset Raman spectroscopy for non-invasive hydration monitoring.

Dehydration can have serious physiological and cognitive consequences that can severely impact performance; this is especially important in high-performance groups such as athletes and military personnel. Monitoring hydration with easy-to-use methods is critical to negate the adverse effects of dehydration, as the effects of inadequate hydration may not be noticed until it's too late to avert dehydration. Current hydration monitoring techniques include biofluid collection (e.g., blood, sweat, urine, etc.) and require laboratory processing, which does not provide rapid, real-time hydration information. Optical vibrational spectroscopy, specifically Raman spectroscopy, can address this lack in *in vivo* hydration monitoring. Further, spatially offset Raman spectroscopy (SORS) allows the probing of interstitial water rather than superficial water, which may not be indicative of tissue hydration. Thus, in the present study, SORS was used to determine the hydration status of exercising individuals. By utilizing a fiber optic probe-based SORS system with dual wavelength excitation, we can capture information regarding macromolecules in the fingerprint region (500-1800 cm^{-1}) and examine water dynamics in the high wavenumber region (2500-3800 cm^{-1}). Spectra were collected from participants before and after exercise, along with the collection of urine and weight change for clinical standard comparisons. Spectra were collected in two anatomical locations and processed for analysis; changes in hydration were quantified by calculating the area under the curve of the water band (3000-3600 cm^{-1}) and the ratio of fully hydrogen bound water to partially hydrogen bound water (AUC 3100-3320 cm^{-1} /AUC 3320-3600 cm^{-1}). The percent change of each metric was calculated to compare the relative hydration change of each participant. Spectral metrics demonstrate considerable inter-subject variability; it is well known that baseline hydration varies greatly, so the heterogeneity seen is not unexpected. Notable changes were observed in spectral metrics when grouping participants based on demographics and clinical standards. Lastly, minimal differences were recorded between the two measurement locations, potentially indicating this technique could be implemented in multiple anatomical locations. SORS for non-invasive *in vivo* hydration monitoring shows great potential for addressing a critical need in hydration monitoring.

(RAM-09.4) Investigation On-Depth Dependent Variation of Accuracy in API Concentration Determination of Tablet using Spatially Offset Raman Scattering Line-Mapping Measurement

Sanghoon Cho¹, Si Won Song², Hoeil Chung¹, Hyung Min Kim²; ¹*Hanyang University*,
²*Kookmin university*

Accuracy of quantitative analysis of API was increased by selecting specific spectra obtained by SORS

In spatially offset Raman scattering (SORS) measurement, the position of the Raman photon collection is away from that of the laser excitation, so when SORS line mapping is performed along a tablet, obtained spectral information in each mapping point represents the sample composition in different depths. For example, a line spectrum acquired farther from the laser illumination point could contain chemical composition corresponding to deeper inside of the sample. Therefore, it would be interesting to examine accuracies of API concentration determination in a tablet when spectra acquired at different line-mapping positions are employed for the analysis. For this study, 30 tablets composed of 4 constituents (naproxen sodium (API), microcrystalline cellulose, lactose hydrate, and magnesium stearate) were prepared and their SORS line-mapping spectra were acquired. Using the line spectra collected from different detector sections, the API concentrations were determined using partial least squares (PLS) and subsequent accuracies were compared in relation with the sampled-depth. Meanwhile, the variation of particle size in a tablet sensitively alters Raman spectral features of the sample and ultimately degrades accuracy. Since the variation of particle size can be recognized using SORS line-mapping as reported earlier, a strategy effectively correcting particle size-induced spectral features and employing the corrected spectra for quantitative analysis expect to be beneficial to secure accuracy. The abovementioned two issues will be presented and discussed.

(RAM-09.5) Raman spectroscopy for measuring systemic physiological hydration in tissue: an analysis of eccrine sweat

Trevor Voss¹, Anita Mahadevan-Jansen¹; ¹*Vanderbilt University*

Fundamental molecular understanding of hydration in tissue for optical spectroscopic, non-invasive, and real-time diagnostics.

There is a need for an accurate, non-invasive, and real-time measurement of physiological hydration.

Current metrics generally seek to measure systemic hydration by measuring the concentration of solutes present in collected fluid samples (e.g., sweat, urine, saliva, blood). Other metrics seek to measure bulk tissue properties in order to back out systemic hydration levels (e.g., skin elasticity, bioelectrical impedance).

In order to create an accurate measurement of systemic hydration, we need to directly measure changes in water content and dynamics in tissue both at the superficial level and with interstitial water deeper in tissue.

High-wavenumber Raman spectroscopy allows us to directly measure water content and its changing dynamics based on its complex molecular environment through its ability to measure changes in hydrogen bonding states in water. Further, the use of spatially offset

excitation and collection fibers allows for measuring both superficially and a few millimeters deep into tissue.

However, the complex molecular environment in human tissue requires a fundamental understanding of the molecular dynamics present in order to accurately assess changes in systemic hydration using Raman spectroscopy. Thus, *Ab Initio* Molecular Dynamic (AIMD) simulations will be made to compute the changing high-wavenumber Raman spectra corresponding to the major hydration related changes in superficial and interstitial water and compared to well controlled experiments.

In order to understand the major contributing factors to the high-wavenumber Raman spectra of superficial tissue hydration, we have focused on measuring changing concentrations of common solutes found in sweat produced by eccrine glands. Sweat cocktails have been created with changing concentrations of urea, lactate, chloride, and sodium ions. Raman spectra are analyzed through peak ratios corresponding to partially and fully hydrogen bound water. We have found a shift away from more fully hydrogen bound schemes to more partially hydrogen bound schemes as the concentrations of solutes is increased.

In order to further understand changing hydrogen bonding schemes water has with its environment in sweat, AIMD simulations of water with changing concentrations of chloride and sodium ions have been made. High-wavenumber Raman spectra have been calculated from this set of simulations and compared to experiment.

22SPECIAL07: Molecular Microspectroscopy and the Molecular Microspectroscopy Laboratory (MML)

Chair: Andre Sommer

(SPEC-07.1) **The History and Beginnings of the MML**

David W. Schiering¹, David W. Schiering¹; ¹*RedWave Technology*

The Molecular Microspectroscopy Laboratory (MML) at Miami University was formed to provide a resource for the scientific community to engage the newly emerging technologies and applications of the molecular microspectroscopies, primarily infrared (IR) and Raman. These technologies now are commonplace and are examples of the most successful, broadly employed, and practical vibrational spectroscopy instrumental methods. Such was not the case in the early 1980s. The formation of MML can be traced to an initial conference in 1983 at Miami University. In attendance were representatives from academia, industry, government, and the instrument companies. Based on feedback from the attendees that molecular microspectroscopy techniques could have wide application and the fact that there was no academic institution specializing in these techniques, the decision was made to acquire funding to form the MML. Some of the industry representatives contributed to the lab formation and the remainder was a loan provided by Miami University. MML was established in 1984 and began operation in the summer. The MML was the first academic

laboratory established that specialized in molecular microspectroscopies. The focus of the lab was research, training of industry, government, and academic researchers, and contract research or analytical services work for hire, utilizing the MML facilities and personnel. MML was a great model for industry-academia collaboration, education of researchers to further contribute to instrumentation development or applications, and training of practitioners, primarily in industrial analytical laboratories. We will provide an overview of the MML formation, the first contributors, the first instrumentation in the lab, and the initial research and training conducted.

(SPEC-07.2) Molecular Microspectroscopy in Art Conservation and Archeology
Patricia L. Lang¹, Pamela A. Smith²; ¹*Ball State University*, ²*Improved Pharma, LLC*

The Molecular Microspectroscopy Laboratory (MML) at Miami University, Oxford, OH was one of the first labs to significantly contribute to the use of infrared, Raman, and visible microspectroscopy for the analysis of art and archeological objects. The presentation will discuss how the lab began in this field and the important role it had in the development of sampling methods and instrumentation design for their use in identifying the chemical composition of historic art works, including the paints on medieval manuscripts and the dyes on archaeological textiles. These techniques offered a keen advantage in the identification of organic materials and in terms of their ease of use. In fact, at the present time, most every large conservation laboratory uses infrared and/or Raman microspectroscopy with sampling techniques developed by the MML.

(SPEC-07.3) Industrial Collaborations with a Focus on Instrument Development
Andre J. Sommer¹; ¹*Miami University*

The Molecular Microspectroscopy Laboratory (MML) was established in 1984 at Miami University in Ohio. The laboratory was the first of its kind in the United States to integrate infrared microspectroscopy, Raman microspectroscopy and optical microscopy in one central facility. Two goals that the laboratory met were to provide industry, academic and government laboratories with state-of-the-art technology to identify microscopic particles and to provide a training facility for those wishing to transfer the technologies to their sites.

Since the laboratory was unique, instrument manufacturers used the lab as a testing site for instrument development and as a demonstration facility. While the lab demonstrated the applications, capabilities and limitations of the technology, it was also key in developing new technologies that pushed the methods forward. This presentation will focus on the development of those new technologies with specific emphasis on FT-Raman microspectroscopy and ATR infrared imaging.

(SPEC-07.4) Kidney and Eye Disease as Studied by Molecular Microspectroscopy
James C. Williams¹; ¹*Indiana University School of Medicine*

This paper will review the use of infrared and other spectroscopic methods for analyzing mineral deposits in pathologies of the eye and the kidneys.

(SPEC-07.5) Molecular Microspectroscopic Analysis of Counterfeit Drugs and Other FDA-Regulated Products

Adam Lanzarotta¹; ¹*US Food and Drug Administration*

The following work will be presented at a SciX 2022 symposium dedicated to scientific achievements made by Miami University's Molecular Microspectroscopy Laboratory (MML). Dr. Lanzarotta joined the MML as a graduate student in the 2005 and became employed at the FDA's Forensic Chemistry Center (FCC) in 2008. Collaborative work between the two laboratories not only contributed to Dr. Lanzarotta's dissertation in 2010 but also to the advancement of FCC's mission for the last several years.

Molecular microspectroscopy has played an integral role for the analysis of forensic casework at the FCC. Examinations using these techniques can often be conducted on the native state of the sample, are generally fast and do not often necessitate significant sample preparation. This presentation will discuss the results of some of the more interesting casework generated at the FCC using molecular microspectroscopic instrumentation. Results from multi-component sample types including counterfeit drugs, illicit drugs, adulterated drugs, drug packaging materials, cross-sectioned animal tissues and autopsy tissues will be presented.

22SPSJ02: Near-Infrared Spectroscopy; Application to Biological and Materials Sciences

Chair: Christian Huck

(SPSJ-02.1) Evaluation of Solid State of Polymers Subjected to Physical Treatments using IR/NIR Spectroscopy

Daitaro Ishikawa¹; ¹*Fukushima University*

The physical properties of starch-based food polymers such as rice flour depend strongly on the internal structure including the particle size, crystallinity, and amylose/amylopectin ratio. Although the strict structure of starch is an advantage in terms of its environmental impact, the designed treatment is necessary for diverse applications. Milling is commonly used as a processing method to produce ideal materials for practical applications, and makes it possible to induce changes in the physicochemical properties of starch. Moreover, in recent year, our research group confirmed that the ordered structure of starch could be modified by water sorption. Thus, the aim of this study was to investigate the internal structure of starch in starch-based polymers based on a physical treatment with non-heating, milling, and water sorption through the structural evaluation of rice flour using SAXS and vibrational spectroscopy. Several types of rice flour samples were gradually prepared by different milling treatments and the samples were subjected to water sorption by eight saturated salt solutions to control within the 0.11–0.98 a_w range. The IR and NIR spectra were measured immediately after removing from the desiccator. SAXS pattern of the samples with low moisture contents subjected to milling yield a band within the 0.4–0.9 nm⁻¹ of the q range owing to a lamellar repeat of starch with an ordered structure in rice flour. The intensity of IR and NIR spectra due to OH bands increased gradually and the position of these bands did not significantly change during water sorption process. On the other hand, the bands around 1745 cm⁻¹ due to lipid on starch was disappeared in high water

activity condition. Thus, bands due to lipid were shown to be potential markers for evaluating structural changes of starch during water sorption process. Moreover the band derived from the COH vibration around 990 cm^{-1} showed no change in wavenumber position around 0.11-0.7 a_w , and it shifted to higher wavenumbers above 0.7 a_w . This result revealed that the water-induced transition of glass to rubber of the starch-based food polymer can be clearly evaluated through vibrational spectroscopy.

(SPSJ-02.2) New Trends in Spectral Preprocessing

Federico Marini¹, Alessandra Biancolillo², Jean-Michel Roger³; ¹*University of Rome La Sapienza*, ²*University of L'Aquila*, ³*INRAE*

Spectroscopic data (or, more in general, experimental data) may be affected by several sources of variability, not all of interest for the specific task the data are collected for. On the other hand, when chemometric tools are applied to the data, very often model building is based on extracting components accounting for a relevant share of the variance in the predictor space, so that all the sources of data variability (wanted or unwanted) will be included in the model: accordingly, if spurious/unwanted variance is still present in the data, it can have a detrimental effect on the resulting model. To, at least partially, reduce or eliminate the effect of such unwanted variability, chemometric model building usually includes one or more pre-processing steps. However, the choice of the best pretreatment or combination of pretreatments to be applied to the data is not always obvious and, in general, a trial and error procedure is followed. In the present communication, a recently proposed strategy, called Sequential Preprocessing through ORThogonalization (SPORT) and based on the idea that the same set of spectra, differently preprocessed could result in a multi-block data, and, accordingly, be processed through dedicated multi-block strategies, will be presented (Roger et al., 2020). It relies on the use sequential and orthogonalized partial least squares regression (SO-PLS; Biancolillo & Næs, 2019), due to the possibility of including/excluding blocks, evaluating their incremental contribution and identifying which matrices carry common and distinctive information). With the occasion, a recently proposed alternative to data normalization called Variable Sorting for Normalization (VSN; Rabatel et al., 2020) will also be introduced.

Biancolillo, A., Næs, T., 2019. The Sequential and Orthogonalized PLS Regression for Multiblock Regression: Theory, Examples, and Extensions. In: Cocchi, M. (Ed.), *Data fusion methodology and applications*, Elsevier, Oxford, 157-177.

Rabatel, G., Marini, F., Walczak, B., Roger, J.-M., 2020. VSN: Variable sorting for normalization. *J. Chemom.* 34, e3164.

Roger, J.-M., Biancolillo, A., Marini, F., 2020. Sequential Preprocessing through ORThogonalization (SPORT) and its application to near infrared spectroscopy. *Chemom. Intell. Lab. Syst.* 199, 103975.

(SPSJ-02.3) Present and Future of Miniaturized NIR-Spectrometers Combined with Challenging Data Management Strategies

Christian W. Huck¹, Krzysztof B. Bec¹, Justyna Grabska¹; ¹*University of Innsbruck*

The ability of straightforward on-site usage, non-destructive analysis of samples featuring wide variety in chemical composition and physical form, while remaining sensitive to the chemical fingerprint is the hallmark of NIR spectroscopy (Bec and Huck, 2020). High performance, sensitivity, reproducibility with low methodological development costs, accompanied by the capacity to perform through-package analysis, makes NIR technique particularly valued in food quality control. In the near future, the problem of quality control will be one of the most important, where the risk is seen two-fold, intentional or accidental; to address both, new, powerful and efficient analytical methods need to be established (Charlebois et al., 2016). NIR spectroscopy appears as one of the most promising analytical frameworks for fulfilling this urgent demand.

In general, the design principles of the NIR instrumentation (spectrometers, optics, cells, sample handling) guarantee a wide area of expansion in the currently rapidly diversifying production and supply chain. The possibility of high sample volume and fiber probe instrumentation enables a fundamental reduction of the necessity of sample preparation. One of the most up-to-date breakthroughs is the sensor miniaturization. Low-cost, portable NIR spectrometers have become reality, and in the next few years, with ultra-miniaturized spectrometers directly integrated smartphone devices being developed nowadays (Bec, Grabska and Huck, 2021). Currently, there are two major trends in advancing NIR spectroscopy in food analysis which are followed in our working group. The first is the development and employment of miniaturized NIR sensors for approaches in the discussed fields. The second trend is the implementation of innovative frameworks for spectra interpretation and calibration, where quantum chemistry provides deeper understanding about the performance of individual spectrometers and chemometric models, respectively.

Beć, K.B., Huck, C.W., Eds. 2020. Advances in near infrared spectroscopy and related computational methods, MDPI. DOI: 10.3390/books978-3-03928-053-7

Charlebois, S., Schwab, A., Henn, R., Huck, C.W. 2016. Food fraud: An exploratory study for measuring consumer perception towards mislabeled food products and influence on self-authentication intentions. Trends Food Sci. & Technol. 50, 211-218. DOI: 10.1016/j.tifs.2016.02.003

Beć, K.B., Grabska, J., Huck, C.W. 2021. Principles and applications of miniaturized near-infrared (NIR) spectrometers, Chem. Eur. J. 27, 1514-1532. DOI: 10.1002/chem.202002838

(SPSJ-02.4) Chemical Information vs. Instrumental Difference in Miniaturized NIR Spectroscopy

Justyna Grabska¹, Krzysztof B. Bec¹, Christian W. Huck¹; ¹*University of Innsbruck*

With simulated NIR spectra it becomes feasible to elucidate sensor sensitivity towards specific chemical information

The ongoing miniaturization of spectrometers creates synergistic effect with the common advantages of near-infrared (NIR) spectroscopy. The combination of portability and direct on-site application with high-throughput and non-invasive way of analysis is a decisive advantage in various analytical scenarios. However, sensor miniaturization requires implementing a number of distinct engineering solutions, and these sensors differ by the key elements used for construction, with impact on their performance and applicability. Narrow spectral working regions of miniaturized instruments limit their ability to measure meaningful vibrational bands, making them selective towards specific chemical constituents. On the other hand, recent advances in theoretical methods enabled accurate simulations of NIR spectra of molecules reaching the size of long-chain fatty acids. Through spectra simulation the location of meaningful variables can be associated with specific molecular vibrations. The sensitivity and specificity of a sensor to chemical information from a given constituent in a complex NIR lineshape of sample can be determined enabling a better-informed design of NIR spectroscopic analysis.

In addition to detailed NIR band assignments, accurately simulated NIR spectra enable new discoveries in physicochemical studies, e.g. the matrix effects can be more easily understood and followed in the data-analytical pathway. Detailed comprehension of the intricate spectral signal opens the pathway to knowledge-based design of the analytical framework by NIR and provides additional measures to optimize its performance.

With the recently provided availability of in silico simulated NIR spectra, it becomes feasible to elucidate in detail the sensor sensitivity towards specific chemical information, and thus gain deeper insight into the critical factors affecting its prediction performance in a given analytical application. This presentation will include brief introduction to the principles and methods, while demonstrating the practical potential of this approach to improve mini-NIR analysis with few examples of relevant case studies, e.g. piperine analysis in black pepper, simultaneous quantification of caffeine and theanine in black tea.

(SPSJ-02.5) Investigation of Reaction Degree of Bio-Coke Formation using Near Infrared Spectroscopy

Yusuke Morisawa¹, Nami Ueno², Hisanori Ozaki¹; ¹*Kindai University*, ²*Kobe University*

Quantitative analysis of BIC formation by NIR spectroscopy and chemometrics

The development of renewable energy and the recycling of waste has been promoted in response to the problems of global warming and rising fossil fuel prices. The use of biomass is attracting attention as one of the new energies, and one of them is biocokes. [1] We have

been developing the Kinda-Biocoke (BIC) that was developed by Ida et al. in 2005. [2] The BIC is a solid biomass sample characterized by high density, high hardness and hydrophobicity, and is expected to be a clean fuel alternative to coal coke and charcoal. There is many studies on physical properties such as hardness and density of BIC have been reported, and applied research such as carbon addition to steel materials is also being actively conducted. However, it is necessary to judge the formation of BIC by hardness measurement, which should destroy the sample for the measurement. Thus, by convention, BIC formation has been determined by the blackness of the product. Miki et al. point out that when woody biomass is used as a raw material, the increase in black parts correlates with the degree of formation of high-density, high-hardness solids. [3] On the other hand, Mizuno et al. found that blackness does not always correlate with density and hardness in herbaceous biomass. In this work we developed the non-destructively analysis for formation of BIC using near infrared (NIR) spectroscopy. In the NIR region, the vibrational transitions at X-H part in different environments are observed as an overlapping band at slightly different wavelengths. A technique called chemometrics is often used for analysis. According to Lambert-Beer's law, what is reflected in the absorption spectrum is the concentration distribution for each type of chromophore. Considering that the degree of the formation reaction depends on the forming temperature in the BIC conversion of wood-based raw material biomass, it is a non-destructive measurement of the NIR spectrum that reflects the distribution of chemical components in the biomass at each temperature.

22PLEN02: FACSS Charles Mann Award for Raman Spectroscopy

(PLEN-02.2) Raman Spectroscopy and Machine Learning for Medical Diagnostics and Forensic Purposes

Igor K. Lednev¹; ¹*University at Albany, State University of New York*

Raman spectroscopy combined with advanced statistics is uniquely suitable for characterizing microheterogeneous samples. Understanding the structure and (bio)chemical composition of samples at the microscopic level is important for many practical applications including material science, pharmaceutical industry, etc. We have recently demonstrated a great potential of Raman hyperspectroscopy for disease diagnostics and forensic purposes. In this presentation, we will discuss the development of a new, noninvasive method for Alzheimer's disease (AD) diagnostics based on Raman spectroscopy of blood, cerebrospinal fluid and saliva. Near infrared (NIR) Raman hyperspectroscopy coupled with advanced multivariate statistics was utilized for differentiating patients diagnosed with Alzheimer's disease, other types of dementia and healthy control subjects with more than 95% sensitivity and specificity. When fully developed, this fast, inexpensive noninvasive method could be used for screening at risk patient populations for AD development and progression.

Raman spectroscopy has already found numerous applications in forensic chemistry providing confirmatory identification of analytes. The technique is non-destructive, rapid

and requires little or no sample preparation. Furthermore, portable Raman instruments are readily available allowing for crime scene accessibility. We have recently demonstrated that Raman microspectroscopy can be used for the identification of biological stains at a crime scene indicating the type of body fluid. In addition, peripheral and menstrual blood as well as human and animal blood can be differentiated. The time since deposition of bloodstain can be estimated up to two years. Most recently, we demonstrated the proof-of-concept for phenotype profiling based on Raman spectroscopy of dry traces of body fluids including the determination of sex, race, and age group of the donor.

22PLEN02: Spectroscopy Magazine's Emerging Leader in Molecular Spectroscopy Award

(PLEN-02.1) Pushing the Frontiers of Stimulated Raman Imaging for Complex Subcellular Bioanalysis

Lu Wei¹, Lu Wei¹; ¹*Caltech*

Innovations in optical spectroscopy and microscopy have revolutionized our understanding in live biological systems at the sub-cellular levels. In this talk, I will present our recent advances in developing and applying stimulated Raman scattering (SRS) imaging, a nonlinear vibrational imaging modality that offers rich chemical information, for specific and highly sensitive investigations of complex biological (i.e. cancer- and neuronal-) systems. First, we integrated Raman spectroscopy and imaging with transcriptomics analysis for metabolic phenotyping in cancer systems. Our subcellular Raman-guided strategy revealed potential new druggable targets that are not present in bulk analysis. Our further integrations with lipidomics and transcriptomics suggest possible underlying regulatory pathways. Second, I will discuss our recent efforts to develop a general sample-expansion vibrational imaging strategy for label-free high-resolution (down to 78 nm) chemical imaging in cells and tissues. With further adoption of machine learning training, we successfully obtained label-free, multi-component, and volumetric prediction of nucleus, blood vessels, neuronal cells, and dendrites in complex mouse brain tissues. We envision this approach will offer an effective and specific way for sub-phenotype profiling especially in large tissue scales.

22AES04: Microfluidic Bioanalysis 1

Chair: Tayloria Adams

(AES-04.1) New Approaches for using 3D Printed Devices for Cell Culture and Analysis

R. Scott Martin¹, R. Scott Martin¹; ¹*Saint Louis University*

Our lab has long been interested in the development of robust microfluidic devices that integrate cell culture and analysis, with electrochemistry being used to detect neurotransmitters/modulators in close-to-real-time. Over the past 5-years, we have transitioned to using Poly Jet 3D printing technology to create microfluidic devices with integrated electrodes for these applications. The first part of the talk will focus on methodology to fully integrate electrode materials into microfluidic devices during the print

process. This approach uses stacked printing (separate printing steps and stage drops) with liquid support to result in devices where electrodes and a capillary fluidic connection are directly integrated and ready to use when printing is complete. Work will be presented on how this approach can be used to fabricate devices with integrated electrodes as well as devices for microchip electrophoresis. A different printing approach will be described for an in-line microfluidic device with amperometric detection, one incorporating a three-electrode set-up that is made possible by threading electrodes into a 3D-printed flow cell. This in-line system can be integrated with a separate mode of detection downstream from the electrochemical flow cell by addition of a mixing T for introduction of reagents for chemiluminescent detection of ATP (via the luciferin-luciferase reaction). This results in a single 3D-printed device that can be used to detect the release of nitric oxide (NO) and ATP, nearly simultaneously, by amperometry and chemiluminescence, respectively. Use of this device to measure NO and ATP release from red blood cells will be described. Finally, culturing cells directly on 3D printed materials is an issue that has not been extensively studied. We will detail recent results from studies designed to understand the effect of different cleaning methodologies on the culture of cells, including methods to minimize leaching of uncured materials into the cell media. This, and approaches to integrating TEER measurements for flow-based culture studies, will be described.

(AES-04.2) Microengineered Platforms to Culture and Measure Signaling within Organs of the Gut-Brain-Immune Axis

Ashley E. Ross¹, Ashley E. Ross¹; ¹*University of Cincinnati*

Communication along the gut-brain-immune axis is vital for maintaining health yet remains difficult to probe with precise temporal and spatial resolution. Our lab develops electrochemical and microfluidic methods, to close this critical gap in measurement science, to probe neurochemical signaling within and between the brain, gut, and immune system. We have developed microengineered platforms that have provided us an experimental platform to probe neurotransmitter signaling in multiple organs simultaneously *ex vivo* during communication, to significantly improve *ex vivo* intestinal slice culture, and to study focal ischemia in the brain. We have coupled these novel platforms with fast-scan cyclic voltammetry (FSCV) recording which enables real-time neurochemical sensing within *ex vivo* tissue slices on-chip. The combination of advanced microfluidic culture platforms with real-time sensing has enabled exquisite insight into the mechanisms of neurotransmitter-regulated signaling in organs along the gut-brain-immune axis. Specifically, we have used these approaches to investigate neurochemical signaling during gastrointestinal inflammation and even neuroprotective responses in the brain during focal ischemia. This talk will highlight some of our recent work in this area on developing microfluidic culture systems for bioanalysis and the application of these methods to study neurotransmitter-regulated signaling in organs of the gut-brain-immune axis.

(AES-04.3) Dielectric Characterization of Ductal Adenocarcinoma Using Murine PyMT+/- Model

Raphael O. Oladokun¹, Soumya Srivastava¹, Timothy Eubank¹; ¹*West Virginia University*

This work will lead to the development of low-cost cell sorting technology for breast cancer.

The growing characteristic of a cancer cell is one of the features that encourage the use of the dielectrophoresis technique to manipulate and carry out electrokinetic separations of normal or healthy cells from cancer cells and vice versa, as they tend to behave differently under a non-uniform electric field. In this paper, we use dielectrophoresis techniques to distinguish the distinct stages of tumor progression.

Animal models are powerful tools to analyse the mechanism of the induction of human breast cancer. This proposal applies transgenic technology in mice (MMTV-PyMT model) to study mammary cancer progression between 4-14 weeks. Our **overall objective** is to develop a diagnostic tool that detects early stages of breast cancer via a non-invasive label-free electrokinetic technique, dielectrophoresis (DEP). This will be achieved by probing the electrical properties of the peripheral blood mononuclear cells (PBMCs) from the whole blood and primary tumor sources of MMTV-PyMT mice at weeks 4 (stage I) and 12+ (stage IV) of infiltrating ductal adenocarcinoma on a microfluidic platform. The **central hypothesis** of this research is that the changes triggered in the subcellular components, such as the cytoskeleton, lipid bilayer membrane, cytoplasm, focal adhesion proteins, and extracellular matrix (ECM) at the onset of carcinoma regulate dielectric (conductivity, σ , and permittivity, ϵ), thus affecting the bioelectric signals that aids in the detection of breast cancer. This hypothesis is developed based on our preliminary published data demonstrating: 1) unique dielectric properties of PBMCs under healthy and early stages of infiltrating ductal adenocarcinoma (ADCs), and 2) label-free sorting [1]. The results obtained here will identify the bioelectric signals that regulate human adenocarcinoma cells. This novel tool is label-free, rapid (~2 min.), and low-cost cell sorting technology that detects early and late stages of breast cancer. This work will lead to preclinical development and future clinical trials of the developed detection platform.

Reference:

1. Adekanmbi E. O., Giduthuri A. T., Srivastava S. K.; "Dielectric characterization and separation optimization of infiltrating ductal adenocarcinoma via insulator-dielectrophoresis," *Micromachines*, **2020**, 11(4), 340.

(AES-04.4) Construction of Microfluidic Electrochemical Cell packed with a Zirconium MOF for sensitive detection of PFOA in Source Water

Zhenglong Li¹, Maryom Rahman¹, Abhishek Kumar², Robbert J Elsingerhorst², Joshua M Torgeson², Julian Schmid², Charmi Chande¹, Radha Kishan Motkuri², Sagnik Basuray¹;
¹New Jersey Institute of Technology, ²Pacific National Northwest Lab

Rapid detection of PFOA at water source is critical for the protection of water resources.

It is well-known that Perfluorooctanoic acid (PFOA) is one of the most dominant environmental contributors. The highly stable carbon-fluoro (C-F) skeletons enable PFOA molecules to exist in nature, especially in the water sources for a long time (half-life > 92 years). Therefore, detecting PFOA levels in the water matrix is a critical topic. Previous work demonstrates that Zirconium (Zr) based metal-organic frameworks (MOFs) have shown considerable affinity to PFOA molecules. In addition, electrochemical impedance spectroscopy (EIS) as a rapid and sensitive detection method (based on measuring the impedance changes at the electrode/solution interface) is perhaps the most frequently used technique in investigating affinity-based transducers. This work applies Zr-based MOF in a non-planar interdigitated microelectrode-based microfluidic electrochemical cell (NP- μ FEC) as an integrated sensing platform for PFOA molecules. Here, the NP- μ FEC, as developed in our group, is a novel impedance sensing platform with an enhanced, three-dimensional distributed electric field. To validate the feasibility of Zr-based MOF-packed NP- μ FEC's sensing performance, experiments are conducted with a PFOA concentration (in 0.1X PBS and tap water spiked with PFOA) ranging from 150 to 10 ng/L. The proposed combination of Zr-based MOF and NP- μ FEC can effectively respond to the PFOA molecules, which offers a detection limit lower than the established US Environmental Protection Agency (EPA)'s water contamination level (70 ng/L) for PFOA and can even meet stricter state standards.

(AES-04.5) **Biomimetic Lipid Membranes as Effective Antifouling Interfaces for Sensing in Clinically Relevant Matrices**

Daniel Stuart¹, Caleb Pike², Quan Cheng¹; ¹*University of California Riverside*, ²*University of California, Riverside*

Investigating the antifouling properties of lipid membranes and how they can improve biosensors

Biosensing with clinical samples is important in enabling clinical diagnoses and providing clinicians with tools to monitor and treat disease. However, biosensors are often limited when dealing with complex samples where surface fouling occurs rapidly and significantly impacts their performance. As such in clinically relevant matrices (blood, serum, plasma, saliva, cerebral spinal fluid, and sputum) which are highly complex, many biological molecules are present that can nonspecifically interact with sensing components and compromise sensor's effectiveness. Therefore, methodologies to reduce these interactions are necessary to decrease sensor noise associated with sensor fouling and improve signal and sensitivity. Antifouling surfaces have been demonstrated as a promising avenue to deal with sensor fouling issues. Recently we have demonstrated lipid membranes as a potential biomimetic antifouling substrate. However, the mechanism behind lipid membrane antifouling properties remains elusive. Herein we report a study of lipid and surface charge

effects on membrane formation and antifouling properties, with a focus on understanding the function and mechanism of positively charged ethylphosphocholine (EPC) lipid membranes on a protein A substrate. Utilizing surface-plasmon resonance (SPR), fluorescence recovery after photobleaching (FRAP), and matrix assisted laser desorption ionization (MALDI) we demonstrated the efficacy of lipid membranes for antifouling purposes, as well as identify charge and steric crowding interactions that are critical to effective lipid bilayer formation and suppression of nonspecific interactions from serum. Favorable charge interactions between the biosensor surface and lipid groups are found to be critical to complete lipid coverage and blockage of fouling protein interactions. In addition to demonstrating an exceptionally successful antifouling lipid membrane, we have also identified parameters necessary for abrogation of nonspecific interactions. We believe this biomimetic lipid membrane and its antifouling surface can be applied to other biosensor applications, and the knowledge will be instrumental in development of new antifouling surfaces to match sensor needs in complex bioanalysis.

22ATOM08: General Session

Chair: Mauro Martinez

(ATOM-08.1) The Characterization of Biogenic Selenium Nanoparticles in Edible Mushrooms by ICP-MS and Complementary Techniques

Jörg bettmer¹, Maria Montes-Bayon¹, Andrés Suárez Priede, Mario Corte Rodríguez¹, Zoltan Mester, Kelly LeBlanc; ¹*University of Oviedo*

Various organisms are capable to produce biogenic nanomaterials. Explored microorganisms for this purpose have been fungi, bacteria, algae among others, and they can provide nanoparticles of various compositions, forms, and sizes. Using such green production strategies, some of these materials cover interesting properties with potential applications in biomedicine or in food industry. However, their production and their potential use need to be accompanied by suitable characterization techniques. In this context, inductively coupled plasma-mass spectrometry (ICP-MS) could play an important role, but so far it has been mainly used for the characterization of manufactured nanomaterials.

This presentation intends to discuss analytical strategies to detect and characterize selenium-containing nanomaterials produced in different types of fungi. Based on ICP-MS in the single particle mode the studies showed mainly nanoparticles in the size of range of about 50 to 250 nm. Their concentrations correlated quite well with the total uptake of selenium. For the detection of smaller nanoparticles, other strategies were necessary, as for instance ICP-MS coupled to liquid chromatography turned out to be a suitable tool. In addition, images from transmission electron microscopy (TEM) confirmed the presence of the detected size ranges.

(ATOM-08.2) Lithium Isotope Ratio Analysis of Geological Samples via Atomic Absorption Spectrometry

Dalia Morcillo García-Morato¹, Alexander Winkelmann¹, Daniel Frick², Lars Jacobsen³, Silke Richter¹, Sebastian Recknagel¹, Jochen Vogl¹, Ulrich Panne¹, Carlos Abad¹;
¹*Bundesanstalt für Material und -Prüfung (BAM)*, ²*GFZ Helmholtz-Zentrum Germany*,
³*LTB Lasertechnik Berlin*

High-resolution coupled to a graphite furnace atomic absorption spectrometer for the isotopic analysis of lithium.

The high diffusivity and strong kinetic fractionation of Li isotopes can be used to determine timescales of geologic processes, where other geochronometers fall short [1]. Isotopic ratio determination is based on monitoring the isotopic components of lithium by their spin-orbit coupling and its isotopic shift of about 15 pm for the $2^2P \leftarrow 2^2S$ electronic transition around 670.788 nm. In this work, we propose improvements to our previous work [2] by using a higher-resolution double echelle modular spectrometer (HR-DEMON II) coupled to a continuum source graphite furnace atomic absorption spectrometer (HR-CS-GF-AAS) for the isotopic analysis of Li.

The data analysis was carried out by using a decision-tree-based ensemble machine learning (ML) algorithm (XGBoost). A set of samples with ^6Li isotope amount fractions ranging from 0.0004 to 0.99 mol mol⁻¹ was used for the algorithm's training. Subsequently, the procedure was validated by a set of stock chemicals (Li_2CO_3 , LiNO_3 , LiCl , and LiOH) and a BAM candidate reference material, a cathode material (NMC111). Finally, the ML model was applied to determine the isotope ratio of geological samples, including anorthosite, granite, soil, rhyolite, nepheline syenite, and basalt. These geological samples were measured as digested without any further purification step.

Improvements in the optical resolution resolve the lithium isotopic components of the atomic spectra (Fig. 1). In the studied geological samples, were found $\delta^7\text{Li}$ values between -0.5 and 4.5 ‰ with a precision range of 0.50 to 1.3 ‰. In addition, the proposed method was validated with multi-collector inductively coupled plasma mass spectrometry (MC-ICP-MS), and these results are comparable and compatible.

(ATOM-08.3) Metallic Nanoparticle Analysis in Semiconductor Grade Tetramethylammonium Hydroxide Using the PerkinElmer Current 5000 Inductively Coupled Plasma Mass Spectrometer

Aaron Hineman¹, Ruth Merrifield¹, Chady Stephan¹; ¹*PerkinElmer Inc.*

In this talk we will show improvements in nanoparticle detection using the NexION 5000 ICP-MS

Metallic contamination in semiconductor products adversely affects device performance. As line widths on chips decrease, the allowable levels of metal contamination also decrease. To meet the demand for higher yields and performance in wafer substrates, contamination must be minimized on the wafer surface as well as in the substrate itself. The most commonly occurring forms of metal contamination are either transition metals or alkaline elements. Transition metals tend to diffuse through the semiconductor material and aggregate on the surface in various oxide forms. Nanoparticle contamination in the process chemicals can cause damage or shorting between lines in the wafer. In this work we demonstrate the superior Dynamic Reaction Cell (DRC) capabilities and detection power of the NexION 5000 multi-quadrupole ICP-MS to analyze nanoparticulate contamination in tetramethylammonium hydroxide which is a common process chemical in the semiconductor industry. It is used as a basic solvent in the development of acidic photoresists in the photolithography process, and is highly effective in stripping photoresists. In addition it can be used to etch silicon, therefore its purity is of utmost concern.

(ATOM-08.4) Laser-Induced Breakdown Spectroscopy Emission Enhancement from Bacteria on a Silver Thin Film

Emily Tracey¹, Haiqa Arain¹, Steven J. Rehse¹; ¹*University of Windsor*

Modified silver targets to amplify bacterial LIBS spectra.

Laser induced breakdown spectroscopy (LIBS) is a technique whereby time-resolved optical emission spectroscopy is performed on high-temperature laser-induced plasmas to determine the elemental composition of the target. This lab uses LIBS to identify and classify bacterial pathogens based on differences in the concentrations of inorganic elements in the membrane of bacterial cells. This rapid classification will be used for diagnosing pathogenic bacteria in clinical specimens. Preliminary work using silver microparticles deposited on a nitrocellulose filter underneath the bacterial cells showed promising enhancement of the bacteria's LIBS emission spectra. This motivated work to increase the uniformity of deposited silver for an improved ablation surface and to prevent the silver deposition from being disrupted by the laser shots. A 60 mJ 1064 nm pulsed laser focused onto a rotating silver foil target in a 10 mTorr evacuated environment sputtered a highly uniform silver thin film onto a nitrocellulose filter. Experiments were performed to locate the filter in a position to sputter the most uniform film across the 9 mm filter diameter. Sputtering times from 1 minute to 20 minutes were investigated. Uniformity was determined with LIBS and scanning electron microscopy.

The silver filters were removed from the vacuum sputtering chamber and ablated in an atmospheric pressure argon environment to acquire LIBS spectra. Analysis of the LIBS spectra exhibited decreased shot-to-shot variation in the silver LIBS intensity between subsequent laser pulses when compared to previous experiments on microparticle covered filters. Bacteria specimens were deposited on a silver filter using a centrifugation

concentration device. Analysis of the LIBS spectra from bacteria deposited on filters created with a sputtering time of greater than 15 minutes showed a 50-100% increase in the magnesium and calcium ion emission intensity, while neutral element emission intensity decreased, indicating an increase in plasma temperature. While overall LIBS emission enhancement was observed for a *S. aureus* sample deposited on a silver filter compared to an empty filter, no statistically significant increase in the signal to noise ratio was found. Work is ongoing to investigate other silver deposition methods including pulsed laser deposition in a non-vacuum environment to create nanoparticles.

(ATOM-08.5) Parametric Optimization and Benchmarking of the Liquid Sampling Atmospheric Pressure Glow Discharge Ionization Source Coupled to an Orbitrap Mass Spectrometer for the Analysis of Plutonium

Joseph V. Goodwin¹, Benjamin T. Manard², Brian Ticknor², Paula Cable-Dunlap², R. Kenneth Marcus¹; ¹*Clemson University*, ²*Oak Ridge National Laboratory*

This is the first reported analysis of plutonium using the LS-APGD microplasma source.

The detection of plutonium during uranium isotope ratio analysis is of utmost importance to the nuclear safeguarding community. When coupled to a benchtop Orbitrap high-resolution mass spectrometer (MS), the liquid sampling-atmospheric pressure glow discharge (LS-APGD) microplasma ionization source has been proven to be an effective and powerful analysis technique for uranium isotope ratio measurements, with the potential to be deployed in the field. The combination of high-resolution mass spectrometry coupled with the LS-APGD has the potential to dramatically reduce the complex sample manipulations required for traditional analysis techniques employed for uranium isotope ratio determinations, specifically thermal ionization mass spectrometry (TIMS) or inductively coupled plasma-mass spectrometry (ICP-MS). Leveraging the high resolution provided by the Orbitrap MS, eliminates the need for pre-analysis separations to remove isobaric interferences. In addition, the LS-APGD operates with lower solution flow rates and has less gas consumption than traditional isotope ratio determinations, leading to a more practical field-deployable analytical solution. To widen LS-APGD applicability and increase its functionality in nuclear safeguarding applications, the LS-APGD has been optimized for the analysis of plutonium. Fortuitously, plutonium exists as PuO_2^+ , like uranium, which exists as UO_2^+ when ionized by the microplasma, allowing for uranium and plutonium to be analyzed concurrently using a narrow range of C-trap injections into the orbitrap, which has been found to increase sensitivity. The optimization and benchmarking of the LS-APGD/Orbitrap combination for the detection of plutonium begins by optimizing in-source collision-induced dissociation (CID) and higher-energy collisional dissociation (HCD) settings to reduce water and other clusters and maximize PuO_2^+ ions. A screening design of experiments was conducted with a dual-electrode LS-APGD source, investigating the effects of counter gas flow, solution flow rate, inter-electrode gap distance, and

current. This work marks the first instance of a design of experiments conducted with a dual-electrode LS-APGD source. The factors found to have the highest correlation for intensity from the screening experiments were further investigated. Once optimal testing conditions were determined, a calibration curve and limit of detection were determined for plutonium. It is believed that the present coupling provides a valuable addition to the nuclear forensics toolbox.

22AWD03: FACSS 2022 Charles Mann Award Symposium Honoring Igor Lednev

Chair: Igor Lednev

(AWD-03.1) 3D SERS Imaging of Nanoporous Gold-Silver Microstructures: Exploring the Formation Mechanism Based on Galvanic Replacement Reaction

Yukihiro Ozaki¹; ¹*Kwansei Gakuin University*

Recently, various 3D SERS substrates have been proposed based on many strategies, such as template metal deposition, ion etching, electron beam lithography, and chemical growth. Some of these techniques possess the inhomogeneous deposition and random aggregation of nanoparticles, which unavoidably leads to high variations in SERS signals. Then, one of our previous studies has proposed an interesting use of nanoporous silver microstructures (AgMSs) as 3D SERS substrates. The galvanic replacement approach enables a scale-up process to create a reproducible product with a high purity of Ag throughout the structure.

We have extended the potential of three-dimensional surface-enhanced Raman scattering (3D SERS) imaging to explore the transformation mechanism of metal contents in the galvanic replacement reaction of 3D nanoporous silver microstructures (AgMSs). The galvanic replacement reaction between AgMSs and Au³⁺ takes place in a saturated sodium chloride solution at room temperature. Owing to the higher reduction potential of Au³⁺ than that of Ag⁺ the galvanized gold–silver microstructures (Au-AgMSs) with the different mole ratios of Au³⁺ and AgMSs were spontaneously fabricated. We developed 3D SERS images of AgMSs and Au-AgMSs using SERS signals of para-aminothiophenol (PATP). The Ag distribution in the microstructures was examined by microscopic and spectroscopic techniques to investigate the structural and morphological changes. 3D SERS profiles support the existence of the atomic diffusion process on the Ag template surface, corresponding to previous studies that the active site of the galvanic replacement reaction presents and forms a small hole for further replacement reaction. This process takes place simultaneously with the galvanic replacement reaction from the Ag surface to the interior of the structure. Accordingly, the transformation of Ag nanoparticles inside the microstructures can be observed in the 3D SERS profile, which cannot be obtained directly from other nondestructive techniques. Moreover, we discussed the additional information about the stability of AgMSs against the atmospheric oxidation of silver metal for a critical selection of using it as 3D SERS substrates.

(AWD-03.2) Innovative Bioanalytical Raman Spectroscopic Sensors Concepts

Jürgen Popp¹; ¹*Leibniz Institute of Photonics Technology*

Bioanalysis requires new detection methods offering molecular specificity, high sensitivity, short detection times and most importantly on-site usability in form of an automated point-of-use approach. In this context Raman spectroscopy is particularly noteworthy since it offers all of the aforementioned features needed to rapidly characterize biomolecules and complex biological specimen without the need of complex sample preparations steps. The combination with chip-based sampling approaches and/or fiber-based probe designs together with modern artificial intelligence based spectroscopic data analysis routines allows for point-of-use Raman concepts covering the entire process chain from sampling to the final result. Here, we introduce novel concepts of on-site / point-of-use Raman spectroscopic sensors concepts for a broad variety of bioanalytical applications: (I) bed-side Raman point-of-care sensors for microbial analysis (e.g. rapid detection of pathogens and their antibiotic resistance pattern together with host response, detection of pathogens in food); (II) cavity enhanced and fiber enhanced Raman sensors for on-site environmental (gas) and drug monitoring; (III) linear and non-linear Raman fiber probes for intraoperative histopathological tissue screening; (IV) surface enhanced Raman spectroscopy (SERS) and surface enhanced IR absorption (SEIRA) sensors for ultrasensitive bio analysis (e.g. detection of antibiotics or disease metabolites in body liquids, forbidden substances in food or metamaterial concepts for chiral biosensing).

Acknowledgements

Financial support of the EU, the "Thüringer Ministerium für Wirtschaft, Wissenschaft und Digitale Gesellschaft", the "Thüringer Aufbaubank", the Federal Ministry of Education and Research, Germany (BMBF), the German Science Foundation, the Fonds der Chemischen Industrie and the Carl-Zeiss Foundation are greatly acknowledged

(AWD-03.3) The Challenges of Translating Research Raman in to a Dedicated Analyzer for Use by Non-Spectroscopists

Andrew Whitley¹, Linda H. Kidder¹; ¹*HORIBA Scientific*

The task of translating research spectroscopy into solution-focused analyzers that address important analytical challenges is not straightforward. Just as with interpreting a language, where the process of translation requires a deep knowledge of both languages, the process of translating *research* requires expertise both in the analytical technique, as well as a deep understanding of the problem that needs to be solved. It does not matter whether it is for life sciences or material development, or whether spectroscopy is to be used for quality assurance of medicine, production monitoring or disease diagnosis - if the problem that requires a spectroscopy solution is not completely understood from all requirements, then the solution will fail. This is seen in the real-world, when a spectroscopic technique in a research setting seems perfect to address an analytical problem, but turns out to completely impractical in the environment for which the analyzer is intended.

This talk will, with a number of examples, review the many applications and business development challenges that slow the practical application of spectroscopy to solving dedicated problems. Ironically the first mistake that often gets made is selecting the wrong problem to solve with spectroscopy, or choosing the wrong spectroscopy to try to solve the problem. There has got to be a clear value proposition not just compared to other spectroscopic techniques, but entire constellation of analytical techniques that might solve the problem. Employing the right subject matter experts like clinicians, process control engineers and regulatory bodies at the beginning is critical to ensure that significant time and money is not wasted, all angles of the problem and solution needs are defined and understood. Even when a problem is hugely important, if the practicality of spectroscopy cannot address all mandated requirements of the solution, then the research or product development should not begin.

(AWD-03.4) Characterization of New Drug Modalities with RAMAN and ROA
Rina K. Dukor¹; ¹*BioTools*

(AWD-03.5) Exploring the Supramolecular Chirality of Protein Fibrils Using VCD
Laurence Nafie¹; ¹*Syracuse University*

With the publication of a JACS Communication paper in 2007 it has been known the vibrational circular dichroism (VCD) has unusual sensitivity to the supramolecular chiral structure of protein amyloid fibrils.¹ Since that time, advances have occurred that have elucidated the sense of the supramolecular chirality to the pH of fibril formation and the morphology of the fibrils as determined by scanning electron microscopy.²⁻⁵ These structure correlations are significant in understanding the relationship of fibril supramolecular chirality to the origin many neuro-degenerative diseases such as Alzheimer's and Parkinson's diseases. In this presentation, the fundamental discoveries leading to our present understanding of amyloid fibril chirality will be described with special emphasis on collaborative role of the Igor Ledev lab in the key breakthrough studies.

1. "Vibrational Circular Dichroism Shows Unusual Sensitivity to Protein Fibril Formation and Development in Solution" by Shengli Ma, Xiaolin Cao, Mimi Mak, Adeola Sadik, Christoph Walkner, Teresa B. Freedman, Igor Lednev, Rina K. Dukor and Laurence A. Nafie, *J. Am. Chem. Soc.* **129**, 12364-12365 (2007).
2. "Direct Observation of pH Control of Reversed Supramolecular Chirality in Insulin Fibrils by Vibrational Circular Dichroism" by Dmitry Kurouski, Rosina A. Lombardi, Rina K. Dukor, Igor K. Lednev and Laurence A. Nafie, DOI:10.1039/C0CC02423F, *Chem Comm.* **46**, 7154-7156 (2010).
3. "Is Supramolecular Chirality the Underlying Cause of Major Morphology Differences in Amyloid Fibrils" by Dmitry Kurouski, Xuefang Lu, Ludmila Popova, William Wan, Maruda Shanmugasundaram, Gerald Stubbs, Rina K. Dukor, Igor K. Lednev and Laurence A. Nafie, DOI:10.1021/ja407583r, *J. Am. Chem. Soc.* **136**, 2302-2312 (2014).
4. "Rapid Filament Supramolecular Reversal of HET-s (218-289) Prion Fibrils Driven by pH Elevation" by Maruda Shanmugasundaram, Dmitry Kurouski, William Wan,

Gerald Stubbs, Rina K. Dukor, Laurence A. Nafie and Igor K. Lednev,
DOI:10.1021/acs.jpcc.5b04779, *J. Phys. Chem. B*, **119**, 8521-8525 (2015).

5. “Origin of Enhanced VCD in Amyloid Fibril Spectra: Effect of Deuteriation and pH” by Marketa Pazderkova, Tomas Pazderka, Maruda, Shanmugasundaram, Rina K. Dukor, Igor Lednev and Laurence A. Nafie, DOI: 10.1002/chir.22722, *Chirality* **29**, 469-475 (2017).

22BIM01: A New Stream of Intelligent Measurements and Data Science Part 1

Chair: Katsumasa Fujita

Co-Chair: Ioan Notingher

(BIM-01.1) On-the-fly Raman Microscopy with Guaranteeing Accuracy Using Reinforcement Learning II: Experiment

Katsumasa Fujita¹; ¹*Osaka University*

Raman microscopy has been expected as a label-free biomedical diagnosis method with improved sensitivity and accuracy due to its molecular detection and analysis capability. However, the measurement time required for Raman microscopy has been hindering the application in practice. In this research, we developed a Raman microscope system using programmable illumination, which can measure Raman spectra simultaneously at arbitrary positions in the sample and determine the positions of following Raman measurements that increases the diagnosis accuracy by on-the-fly Raman spectrum analysis. We developed the programmable illumination system using a liquid-crystal-based spatial light modulator, and the illumination pattern was determined by the bandit Raman analysis algorithm that utilizes results of Raman spectrum analysis using machine learning and provides a diagnostic result at each position and exposure in the sample. We used mixtures of polystyrene/PMMA beads as models for tissue diagnosis and demonstrated that the on-the-fly Raman microscopy drastically improved the diagnosis speed without sacrificing the accuracy as predicted by the theoretical simulations.

(BIM-01.2) Selective Sampling Raman Spectroscopy for Biomedical Applications

Ioan Notingher¹; ¹*University of Nottingham*

Many biomedical applications of Raman spectroscopy require acquisition of both spatial and spectral information from the sample. As typical Raman spectroscopy measurements require long acquisition time, scanning large tissue specimens at the high spatial and spectral resolution required for achieving clinically relevant accuracy remains a challenge. We present techniques based on selective sampling Raman micro-spectroscopy that have the potential to overcome these challenges. These techniques rely on selecting the optimal locations for sampling Raman spectra to target a specific chemical species based on spatial correlation of tissue. This can be implemented in real-time (sampling points allocated on-the-fly based on real-time analysis of measured spectra) or sequentially based on dual-modality approaches where faster and high-resolution optical modalities are used to obtain spatial information. Selective sampling reduces the time required for diagnosis cm-scale tissue specimens by minimising the number of Raman spectra acquired while maintaining sufficient spatial information to meet the clinical need. We will present latest clinical results

based on these technique for intra-operative diagnosis of surgical margins in cancer surgery based on optimised prototype instruments.

(BIM-01.3) Intelligent Image-Activated Cell Sorting 2.0

Keisuke Goda¹; ¹*The University of Tokyo*

A fundamental challenge of biology is to understand the vast heterogeneity of cells, particularly how the spatial architecture of cells is linked to their physiological function. Unfortunately, conventional technologies are limited in uncovering these relations. In this talk, I introduce a new type of technology known as “intelligent image-activated cell sorting (iIACS)” that performs real-time image-based sorting of cells at an unprecedented rate of >2000 events per second. This technology integrates high-throughput cell microscopy, focusing, sorting, and computational analytics on a hybrid software-hardware data-management infrastructure, enabling real-time automated operation for data acquisition, data processing, intelligent decision-making, and actuation. It extends beyond the capabilities of traditional fluorescence-activated cell sorting (FACS) from fluorescence intensity profiles of cells to multidimensional images, thereby enabling high-content sorting of cells or cell clusters with unique spatial chemical and morphological traits. In this talk, I show several unique applications of the technology and introduce its recent upgrades.

(BIM-01.4) Optimizing Microscopy and Spectroscopy Instrumentation for Data Throughput

Chris Rowlands¹; ¹*Imperial College London*

Many problems in microscopy and spectroscopy (speed, resolution, image contrast) are inherently data throughput problems. Increasing an instrument's speed means finding a way of gathering data more quickly. Improving resolution inherently requires gathering more data. Image contrast often requires imaging with more spectral channels - another data throughput problem.

In this presentation I will highlight work in my lab focussed on increasing data throughput of a variety of different optical systems, including super-resolution structured illumination microscopes, hyperspectral imaging systems (including Raman instruments), multiphoton microscopes and more. The emphasis will be on the practicalities of building these systems: the advantages they bring, the (primarily biomedical) applications they were designed for, the key innovations behind them, and some discussion of the difficulties encountered during their creation.

(BIM-01.5) Ramanomics - A New Raman Microscopy Based Omics Technology For Quantitative Analysis Of Biomolecular Composition In Live Cells And Tissues

Andrey Kuzmin¹, Alexander Rzhetskii², Artem Pliss¹, Paras Prasad¹; ¹*SUNY, University at Buffalo*, ²*Thermo Fisher Scientific*

New single organelle Omics based on Raman microscopy (Ramanomics) is presented

Raman microscopy is one of the most powerful analytical techniques for the characterization of complex biological samples and noninvasive monitoring biochemical processes in situ. This technique uniquely bridges optical microscopy and molecular analysis of subcellular structures or microscopic tissue regions and, therefore, is increasingly demanded in modern biomedical research applications.

Confocal Raman microscopy in combination with a biomolecular component analysis (BCA) algorithm provides new opportunities to study molecular interactions and dynamics in biological systems at sub-micron levels. The core of the BCA analysis is a spectral fit of the measured Raman spectrum by the linear combination of the weighted spectra of the basic biochemical components such as proteins, lipids, nucleic acids, polysaccharides, etc. The optimized spectral weights directly yield the concentrations of the biochemical components and important parameters of microlipidomics. The combination of micro-Raman/BCA technique, together with powerful bioinformatics approaches provide basis for the Ramanomics, a new bioanalytical technique with unprecedented capabilities of systemic molecular analysis in cellular organelles. In this presentation we review the current progress in Ramanomics for analysis in live cells and further perspectives of this technique for study of biological tissues. Our data show that Ramanomics can provide the link between the biomolecular composition and physiological or pathological cell functions to elucidate the mechanisms of cellular regulation and disease development.

A step-by-step procedure for implementing the Ramanomics platform with the use of an upright confocal Raman microscope is provided.

22CHEM04: Chemometrics and Food Safety

Chair: Mengliang Zhang

(CHEM-04.1) Raman Spectroscopy with On-board Chemometric Models and Library Spectral Matching for Plasticizer Identification

Betsy Jean Yakes¹, Josh Moskowitz², Luke K. Ackerman¹, Kristen Reese¹, Timothy Begley¹, Katherine Carlos¹; ¹*U.S. Food and Drug Administration*, ²*University of Maryland, Joint Institute for Food Safety and Applied Nutrition*

Chemometrics play an increasingly large role in food safety, and there are many examples of portable vibrational spectroscopy with chemometric analysis for food products such as meat and honey authentication, grains and coffee region of origination determination, milk powder and spices adulteration detection, and edible oils and dietary supplements quality evaluations. Additionally, advanced data processing is being applied to food contact materials in order to understand composition and quality as well as support regulatory endeavors. One example is for polyvinylchloride (PVC) evaluation for understanding use in and composition of tubing and bottle cap gaskets. To enable the softness and flexibility that is necessary in these applications, plasticizer compounds are added to the PVC during formulation. In order to identify the plasticizer used and support the food industry and

regulators, our lab developed two rapid, portable Raman spectroscopy methods with advanced library matching and chemometrics models that may be able to be used outside of the laboratory. In this presentation, we will highlight a 785 nm Raman spectrometer with hierarchical chemometric modeling for plasticizer identification and a 1064 nm Raman spectroscopy method using an advanced library matching routine for chemical identification. Finally, a blinded evaluation of 15 food production tubing samples and 26 bottle cap gaskets will be highlighted with insights shown into use trends over recent years and how lab-based, confirmatory GC-MS and rapid DART-MS can create a sampling methodology for robust plasticizer detection.

(CHEM-04.2) Chemometrics-Based Correlations Between Chemical Changes and Biological Effects in Food Safety Research

Chi Chen¹, Qingqing Mao, Jieyao Yuan; ¹*University of Minnesota Twin Cities*

Chemical toxicants and pathogenic microbes are the sources of concerns in food safety. Chemometrics as an effective tool to probe the chemical differences between normal and spoiled of food as well as the metabolic changes in microbial pathogens under growth and challenges is illustrated by two case studies. In the first case study, chemometric profiling of aldehydes in six oxidized soybean oils produced under different thermal stress conditions revealed the correlations between the formation of individual aldehydes and the temperatures and durations of heating. Further correlation analysis on the quality markers, the concentrations of aldehydes, and the growth performance of pigs and broilers fed these oils, showed that *p*-anisidine value and C9-C11 unsaturated alkenals had the best inverse correlation with the growth performance of broilers and pigs. In the second case study, the bactericidal and bacteriostatic effects of intense pulsed light (IPL) were examined by the multivariate modeling of *E. coli* metabolome. The IPL-elicited time- and dose-dependent reductions in colony-forming units (CFU) and morphological changes of *E. coli* were correlated with the metabolic changes. The results revealed a cascade of events that might be initiated by the degradation of quinone electron carriers and then followed by oxidative stress, disruption of intermediary metabolism, nucleotide degradation, and morphological changes. These case studies highlight the efficiency of chemometrics for characterizing complex chemical changes in foods and metabolic changes in pathogenic microbes.

(CHEM-04.3) Chemometrics in Spectral Data Applied to Food Quality, Safety and Authenticity

Mohammed Kamruzzaman¹; ¹*University of Illinois at Urbana-Champaign*

Food industry and supply chain need low-cost, non-destructive, rapid, and accurate sensing technologies for the quality, safety, and authenticity of food and food products. NIR spectroscopy or hyperspectral imaging is one of the most widespread modern analytical techniques for sensing food quality, safety, and authenticity. The technology has strong perspectives for further development due to advancements in optics, computing power, and machine learning. The technique has several advantages such as rapid, precise, non-destructive, and multi-analytical; hence, several constituents can be predicted simultaneously from the same spectrum. One of the advantages of these sensing techniques is the wealth of

data. These data are multivariate due to a large number of data variables at each spectral band. Chemometrics analysis is thus an indispensable part of these analytical technologies. It is required to appropriately extract meaningful information from the spectra to correlate with food quality, safety, and authenticity parameters. Chemometrics analysis has emerged as an essential analytical tool in many applications in the last few decades. The reason for the considerable interest in chemometrics is that the technique is fast and cheap, not 100% accurate, but accurate enough for many real analytical applications. Although there are many challenges in implementing these spectral technologies for real-time implementation, it is expected to become one of the most promising analytical tools with the fusion of chemometrics data analysis for food quality, safety, and authenticity. In this talk, different chemometrics strategies applied to spectral data for food quality, safety, and authenticity will be discussed. The challenges and future trends of NIR spectroscopy and hyperspectral imaging will also be addressed.

S H B W 3 9 9 3

(CHEM-04.4) Metabolomic Study of Wild American Ginseng and Cultivated American Ginseng Roots by UHPLC-HRMS and Chemometrics

Roderick W. Moore¹, Mengliang Zhang¹, Ying Gao¹, Jianghao Sun², Zhihao Liu²; ¹*Middle Tennessee State University*, ²*Food Composition and Methods Development Laboratory, BHNRC, ARS, USDA*

Wild American ginseng (*Panax Quinquefolius* L.) is a pharmacologically and agriculturally important crop native to Tennessee. The wild roots have greater market value and contain a higher level of active pharmacological compounds (i.e., ginsenosides) compared to their cultivated counterpart. The primary goal of this project is to study the phytochemical profiles of wild American ginseng roots by using an untargeted reverse-phase ultra-high performance liquid chromatography coupled to a high-resolution mass spectrometer (RP-UHPLC-HRMS) based metabolomic approach. Twelve wild American ginseng roots of 4 different ages (i.e., 7, 9, 10, and 12 years old) were collected and compared with the cultivated American ginseng roots from different sources. Chemometric methods such as analysis of variance principal components analysis (ANOVA-PCA) and partial least-squares discriminant analysis (PLS-DA) were used to analyze the variance induced by the experimental factors such as wild vs. cultivated ginseng and age effect. The browser-based metabolomic platforms were also used to process the RP-UHPLC-HRMS data. The data matrix was further deconvoluted into the ginsenoside relevant and non-ginsenoside relevant matrices. Feature compounds will be identified, and these biomarkers would allow for a better understanding of the biochemical and biological significance occurring between American ginseng grown via traditional cultivation methods and American ginseng found in its natural habitat.

(CHEM-04.5) In-Field Assessment Of Flavor Traits In Tomatoes Using Portable Scanner

Shreya M. Nuguri¹, Celeste Matos¹, Peren P. Aykas¹, Luis E. E. Rodriguez-Saona¹; ¹*The Ohio State University*

Handheld system assist tomato breeders to assess the effects of breeding techniques on fruit's flavor.

Justification: Tomatoes are the largest produced fruits in the world. Due to their rising demand, tomato breeders aim to produce disease-resistant, better yield and longer shelf-life tomatoes. However, it has been observed that organoleptic qualities get overlooked during these breeding efforts; hence, it becomes crucial to monitor flavor traits of tomatoes. Current analytical techniques are time-consuming, labor-intensive, and expensive. Advancements in miniaturization of spectrometers offers high-throughput, in-field capabilities for assessment of new breeding material. The numerous growths in these portable spectrometers have made it suitable as rugged analyzers for on-spot analysis.

Objective: To optimize the selection capabilities during the breeding of tomatoes by developing predictive algorithms for rapid characterization of flavor traits using handheld Near Infrared (NIR) scanner.

Methods: Tomato varieties (Roma, round, grape, cherry) at different ripening stages (n=400) were supplied by a farm in Florida (2020-22). Two different approaches were used for collecting spectral data from the samples: juice and intact fruit. Spectra were collected using handheld FT-NIR scanner that utilizes monolithic opto-electro-mechanical structure. Official analytical methods were used to determine the quality traits which included Sugars (Glucose and Fructose), Brix, Titratable Acidity, Acids (Ascorbic and Citric) and Lycopene. Partial least square regression (PLSR) was used to generate prediction models.

Results: PLSR showed good correlation coefficients ($r_{val} > 0.73$) for models developed using tomato surface spectra, while juice spectra gave a higher correlation coefficients ($r_{val} > 0.91$) and an excellent predictive performance for all quality traits. Multiple quality traits were simultaneously determined based on unique spectral fingerprints, by using a single drop of sample providing fast (10 sec) measurements and minimal sample preparation.

Significance: Novel handheld systems may provide the tomato breeding industry with a rapid method to evaluate flavor characteristics of tomatoes allowing them to assess the effects of breeding technique on the organoleptic parameters with a great analytical flexibility since the unit can be easily carried for field applications.

Keywords: Tomatoes, Flavor traits, handheld FT-NIR, PLSR

22CTP/EARLY03: SAS Organized Session: Navigating Challenges to Achieve Success as an Early Career Spectroscopist, Part 1

Chair: Fay Nicolson

Co-Chair: Andrew Whitley

(CTP-03.1) My Transatlantic Transition from Post-Doc to Professor

Samuel Mabbott¹, Samuel Mabbott¹; ¹*Texas A&M University*

Transitioning into an academic career can be simultaneously exciting and daunting. As I committed to a transatlantic transition from post-doc to professor, it took me a while to realize just how ‘green’ I was when it came to the broad number of responsibilities a professor has. Now, 4 years into the role I feel there is still much for me to learn but also several tips and snippets of advice I can offer to others who picture themselves as dedicated to pursuing an academic career. During my talk I will give a brief overview of my pathway to academia, touch on the three vital academic commitments; research, teaching, and service, and provide you with some personal tips that I learned to progress to where I am today.

(CTP-03.2) Academic-Industrial Collaboration: Bringing New Imaging Frontiers for Pharmaceutical Processes.

Prabuddha Mukherjee¹, Prabuddha Mukherjee¹, Michael Olszowy; ¹*Sartorius Stedim Biotech*

As with many scientific disciplines, spectroscopists encounter a broad range of challenges as they identify and pursue the research questions, they would like to address within their chosen discipline. Perhaps most notable are the tasks of gathering and managing resources to address constant pressure to sustainably fund their research as their careers emerge. For early training, resources to support scientific research are available and are relatively easily accessed within academic environments. This training forms the base of emerging professional visibility through publications and participation within the scientific community. As careers progress, pressure to maintain relevance through consistent publication and funding remains. On the other hand, funding for research in corporate/industrial settings can be substantial and is typically more stable due to the nature of commercial entities that are not as prone to funding fluctuations from political environments and other pressures. However, in the continuum of applied versus basic research, industry tends toward the applied. In this context, academic-industrial collaborative research can enjoy benefits from both sides - industrial, commercial funding directed to more basic research. In my presentation I will speak about one such academic-industrial collaborative endeavor that was successful, academically fulfilling and was instrumental in securing next level employment.

(CTP-03.3) Scientists at P&G

Stefania Perticaroli¹, Ariel Lebron¹, Stefania Perticaroli¹; ¹*The Procter and Gamble Company*

We are two vibrational spectroscopists and Senior Scientists at P&G, but with different career journeys. In this Early Career talk, we would like to share our path, describe how each of us had moved from academia to industry, and our day-to-day work at a multinational consumer goods corporation. We will provide examples of how we use

spectroscopic analytical tools for R&D applications. This is a story of challenges, reinvention, collaboration, and innovation.

(CTP-03.4) They Do Research at the FDA? How to Survive and Thrive in a Regulatory Research Environment

Betsy Jean Yakes¹; ¹*U.S. Food and Drug Administration*

Research at the Food and Drug Administration (FDA) has always been a key pillar of supporting public health, starting in the early days with Dr. Wiley and his “Poison Squad”. The labs have grown over the years to support the safety of 78% of all food consumed in the US, including everything except for meat, poultry and some egg products. But maybe you didn’t know that the FDA did research? And, if you did, how would you get a job there, and what is it like to have a career in regulatory research? This presentation will give a brief history of the FDA along with snapshots from my 15 years of research there, as well as highlight critical moments along such a career path in this environment including finding a postdoc/job, developing a research niche, getting promoted, and balancing a 2-body scientist and growing family during the early days of a career. By the end, you’ll know that although the US food supply is one of the safest in the world, the CDC estimates that 48 million people become ill each year as a result of foodborne illnesses, and the team at FDA, perhaps including you in the future, is hard at work to help bend this curve through having premier original research, advanced methods development, and robust scientific analysis.

(CTP-03.5) Panel & Open Discussion

Ariel Lebron¹, Stefania Perticaroli¹; ¹*The Procter and Gamble Company*

22IR03: Nanoscale Spectroscopy: Advances in Instrumentation

Chair: Andrea Centrone

Co-Chair: Andrew Whitley

(IR-03.1) Emerging Trend in AFMIR: Surface-sensitive Mode on the way to Probe the Depth of a Sample

Ariane Deniset-Besseau¹, Alexandre Dazzi¹, Jérémie Mathurin², Martin Wagner³;

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In the last decade, the AFM-IR technique has progressively become a reference technique for infrared analysis at the nanometric scale. This technique combines the high spatial resolution of an AFM (Atomic Force Microscope) with the vibrational analysis capabilities of infrared spectroscopy. The field of applications is exceptionally vast and covers fields as diverse as molecular biology, polymer science, microbiology, medicine, geology, ancient materials, and astrochemistry [1]. Currently, the AFM-IR measurements are implemented with 3 different AFM modes (contact, tapping, peak force tapping [2]) and allow the analysis of many types of samples in terms of hardness and geometry. More recently, our

team has developed and patented a new acquisition mode [1]. It's using the AFM in its classical contact mode to probe the first tens of nanometers of a sample surface instead of the usual few micrometers. This mode is called 'sensitive surface', and we have high hopes that it will help the AFMIR analysis of samples with nanometric active coating as well as surfaces with wanted or unwanted deposits (contaminant, protective layer, active layers, or oxidative products). More widely, samples for which the substance to be detected is present in small quantity and is located on a substrate, highly absorbing in the same spectral region. During the oral presentation, the general theoretical background, as well as some experimental constraints, will be discussed and results obtained on a co-polymer will be presented and commented on: we clearly observe separate polystyrene (PS) drops of a few nanometers thick in a dense polymethyl methacrylate (PMMA) matrix.

[1] J. Mathurin et al. *J. Appl. Phys.*, 131, 010901 (2022).

[2] A. Dazzi, C.B. Prater, *Chem. Rev.*, 117, 7, 5146–5173, (2017).

(IR-03.2) **Seeing Atoms: TERS in the Atomistic Near-field**

Vartkess A. Apkarian¹; ¹*University of California, Irvine*

(IR-03.3) **Single Molecule Nano-Chemical Imaging and Spectroscopy to Unravel Molecular Structure and Interactions**

Francesco Simone Ruggeri¹; ¹*Wageningen University*

A fundamental objective of modern analytical methods in physics, chemistry and biology is the comprehension of how the physical-chemical state and heterogeneity of biomolecules determine their role in cellular function and disease. However, biomolecules have nanoscale physical dimensions, and their function emerges from a correlation between their chemical and structural properties.

Here, we show the development and application of photothermal infrared absorption nanospectroscopy (AFM-IR) as a real breakthrough for the analysis of heterogeneous biomolecules and their interactions from the single molecule [1], through supramolecular assemblies [2], to the single cells and organism scale [3].

As a major advance in the field, we demonstrate the achievement of single protein molecule detection of infrared absorption spectra and maps by introducing off-resonance, low power and short pulse infrared nanospectroscopy (ORS-nanoIR) [1]. Pushing the sensitivity of AFM-IR to its current limit, we prove the determination of the secondary structure of single proteins molecules and amyloid self-assemblies, which are involved in the onset of neurodegenerative disorders, with similar accuracy as obtained in bulk by FTIR [1-2]. Exploiting this unprecedented sensitivity, we prove the unravelling of the molecular interaction fingerprint of amyloid species with a small molecule capable to prevent the disease in animal models of neurodegeneration [2]. Furthermore, we apply our single-molecule capabilities to study the surface properties of artificial model membranes [5], organic solar

cells [6], and the structure of functional protein self-assemblies as promising candidates for the development of novel class of biocompatible and sustainable biomaterials in bioscience [7].

Overall, our aim is to expand the capabilities of nanoscale vibrational spectroscopy to shed light on the structure-activity relationship of biomolecules for applications in nanomedicine, materials science and biotechnology.

[1] Ruggeri, **Nature Comm.**, 2020.

[2] Ruggeri, **Nature Comm.**, 2021.

[3] Otzen, ..., Ruggeri, **Small Methods**, 2021.

[4] Marchesi, **Advanced Functional Materials**, 2020.

[5] Doherty, **Science**, 2021.

[6] Shen, Ruggeri, **Nature Nanotechnology**, 2020.

(IR-03.4) Chemically Identifying Single Adatoms with Single-bond Sensitivity During Oxidation Reactions of a Polymorphic Atomic Monolayer

Nan Jiang¹; ¹*University of Illinois Chicago*

The chemical interrogation of individual atomic adsorbates on a surface significantly contributes to understanding the atomic-scale processes behind on-surface reactions. However, it remains highly challenging for current imaging or spectroscopic methods to achieve such a high chemical spatial resolution. Here we show that single oxygen adatoms on a boron monolayer (i.e., borophene) can be identified and mapped via ultrahigh vacuum tip-enhanced Raman spectroscopy (UHV-TERS) with ~ 4.8 Å spatial resolution and single bond (B–O) sensitivity. With this capability, we realize the atomically defined, chemically homogeneous, and thermally reversible oxidation of borophene via atomic oxygen in UHV. Furthermore, we reveal the propensity of borophene towards molecular oxygen activation at room temperature and phase-dependent chemical properties. In addition to offering atomic-level insights into the oxidation of borophene, this work demonstrates UHV-TERS as a powerful tool to probe the local chemistry of surface adsorbates in the atomic regime with widespread utilities in heterogeneous catalysis, on-surface molecular engineering, and low-dimensional materials.

(IR-03.5) Nanoscale Spectroscopic Investigations of Core-Shell Nano Particles as Potential Drug Carriers

Volker Deckert¹, Christiane Höppener¹; ¹*Leibniz-IPHT*

TERS and nano-mechanical studies allow tackling the nanoscale composition of individual multi-layered drug carriers

Block copolymers are composed of sequences with distinct functional units. Amphiphilic block copolymers can form for instance stable micelles or vesicles with different properties

of core and shell and potentially even further layers. One particular application could be the delivery of hydrophobic drugs contained in the core while the hydrophilic shell for instance provides solubility. We will discuss a general pathway to investigate such particles using a combination of atomic force microscopy (AFM) based topography and force-distance nano mechanical experiments in combination with tip-enhanced Raman scattering (TERS). The investigated system consist of a hydrophilic polyethylenoxide (PEO) shell linked to a hydrophobic and cross linkable poly furfuryl glycidyl ether-co-tert-butylglycidyl ether (P(FGE-cotBGE)) core. In order to stabilize the micelles commonly cross-linking reactions are used, here a Diels-Alder coupling via bis-maleimide (BMA). Utilizing the freeze fracture technique allows to investigate sliced nanoparticles and consequently distinguish between core and shell. We will discuss how the different techniques allow not only to clearly distinguish between core and shell, but also provides direct information regarding the cross-linking efficiency via the nano mechanical properties of individual micelles. These properties are complemented by structural specific TERS experiments that provide direct evidence regarding the cross-linking yield across a single block copolymer nano particle.

22LIBS04: Molecular

Chair: Michael Gaft

(LIBS-04.1) Features in Molecular LIBS

Christian G. Parigger¹; ¹*Ariel University*

This work focuses on diatomic molecular spectroscopy for diagnosis of plasma, especially for analytical chemistry applications of laser induced plasma. Of primary interest are molecules such as cyanide, CN, aluminum monoxide, AlO, diatomic carbon, C₂, titanium monoxide, TiO, and hydroxyl, OH. Accurate line-strength data are available for modeling of selected molecular transitions that can be measured in plume expansions with time-resolved emission spectroscopy in the near-UV to near-IR spectral regions. Alternate computational modeling is discussed as well, for example, for diagnosis of measured alkaline-earth metal strontium monoxide, SrO, spectra. Analysis of spatiotemporal data recorded with nano-second laser-induced breakdown spectroscopy, LIBS, reveals how expansion dynamics and shock-wave phenomena relate to measured radial distributions, especially for CN that is noticeable within the first few hundred nanoseconds after optical breakdown. Abel inversion of temporally- and spatially- resolved line-of-sight data determines the CN spatial distributions. Computed chemical equilibrium distributions allow one to infer molecular density as function of temperature. Application of fitting routines yields temperature from a molecular spectrum, consequently, one can find the molecular density, for example for the OH molecule. Analysis of C₂ Swan astrophysical molecular spectra has further challenges, but in turn can serve as a guide for chemical analysis of laboratory plasma that contains carbon.

(LIBS-04.2) MLIBS-MLIF Methods for Quantitative and Isotopic Analysis

Lev Nagli¹, Michael Gaft¹, Yosef Raichlin¹; ¹*Ariel University*

MLIBS-MLIF methods for quantitative and isotopic analysis

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The strongest LIBS lines of Be at 313.04 and 313.11 nm are resonant and reabsorbed by the plasma plume, leading to the strong nonlinearity of the calibration curves. Proposed in [1], molecular LIBS (MLIBS) of BeO diminished self-absorption effects but suffers from lower sensitivity. In the next step, we combine (LIBS) with Laser-Induced Molecular Fluorescence (LIBS-MLIF) [2], allowing for an improved BeO limit of detection. A typical example is shown in Fig.1, which compares MLIBS and LIBS-MLIF spectra of Be in Cu/Be bronze. The MLIF (1,1) line is about three times more intense than the strongest MLIBS (0,0) line.

Fig.1 BeO emission in MLIBS and MLIF spectra.

MLIF method was also demonstrated on Sr isotopes determination using blue-green and UV SrO molecules emission.

[1] M. Gaft, L. Nagli, A. Gorychev, Y. Raichlin. Atomic and Molecular emission of beryllium by LIBS. *Spectrochimica Acta B* 182(2021)106233

[2] L. Nagli, M. Gaft, Combining Laser-Induced Breakdown Spectroscopy with Molecular Laser-Induced Fluorescence, *Applied Spectroscopy* 70 (2016) 585–592.

(LIBS-04.3) Molecular Formation in Nebulized Assisted LIPs: Detection of Halogens

Nerea Bordel¹, Cristina Méndez-López¹, Luis Javier Fernández-Menéndez¹, Cristina Gonzalez-Gago¹, Jorge Pisonero¹; ¹*University of Oviedo*

Laser Induced Breakdown Spectroscopy (LIBS) is an analytical technique based on the measurement of the emitted radiation coming from a laser induced plasma created after the irradiation of a sample by a short laser pulse (10^{-15} - 10^{-9} s). This technique is used for the direct determination of the elemental sample composition. LIBS allows, in principle, the detection of any element of the periodic table, although the resulting detection sensitivity depends on the element to be analyzed. It is well known that, in the case of halogens, the detection limits resulting from the measurement of the emission of atomic lines in a laser-induced plasma in air are orders of magnitude higher than those obtained for metallic elements. Among the different analytical methodologies proposed to improve the sensitivity of halogen detection, the use of the emission of the molecule resulting from the

recombination of the halogen with an alkaline earth can be highlighted [1]. However, producing the formation of these molecules in plasmas when samples do not contain the metallic element requires some additional strategy. It has been shown that nebulisation of a Ca-containing solution onto an F-containing sample while LIBS analysis is taking place results in the molecule formation, being possible the detection of the molecular emission [2].

This talk critically reviews the effect that nebulisation has on laser-induced plasmas and its impact for analytical applications. In particular, the effect of nebulisation on the sample surface as well as on plasma properties (excitation temperatures and electronic densities) is evaluated. In addition, the molecular species formed when nebulising different solutions are investigated, as well as their spatial distribution and time evolution. Finally, some applications focused on the quantification of halogens in different samples are presented.

[1] M. Gaft, L. Nagli, N. Eliezer, Y. Groisman, and O. Forni, *Spectrochim. Acta Part B* 98 (2014) 39–47

[2] C. Álvarez-Llamas, J. Pisonero and N. Bordel, *J. Anal. At. Spectrom.*, 32 (2017) 162-166.

(LIBS-04.4) **REE Molecules in LIBS**

Michael Gaft¹, Michael Gaft¹; ¹*Ariel University*

The detection of Rare-Earth-Elements (REE) by LIBS by their atomic and ionic emission in many cases is difficult due to significant spectral interferences from other members of this group and accompanying elements. Another potential detection mode is to use molecular emission. We proved that the molecules of all REE with oxygen appear effective for analytical purposes. The type of the emission spectrum depends on specific element molecular weight and differs for Light and Heavy REE sub-groups. The analytical approach is demonstrated for Gd analysis in ceria (CeO₂) and in permanent B-Fe-Nd magnets.

The presence of molecular emission opens an opportunity for isotopic analysis. We started to research this option. It was found that the width of the molecular emission lines is strongly element dependent. The mostly narrow ones belong to Y, La, Sc, and Lu. All those elements exist in nature or as one stable isotope (Y, Sc) or mostly one stable isotope (La and Lu). Opposite to that, the elements with relatively broad molecular emission contain several stable isotopes. For example, in nature the gadolinium occurs as a mixture of six stable isotopes with close abundances - ¹⁵⁸Gd (24.84 %), ¹⁶⁰Gd (21.86 %), ¹⁵⁶Gd- (20.47 %), ¹⁵⁷Gd (15.65 %), ¹⁵⁵Gd (14.8 %), and ¹⁵⁴Gd (2.18 %). The spectroscopy with resolution of 0.02 nm revealed the absence of spectral splitting in monoisotopic elements, while in Gd for individual emission bands at the strongest transition (0,0 transition, System I) became visible peaking at 461.50, 461.56, 461.52, and 461.70 nm.

At the second stage, we applied Molecular Laser-Induced Fluorescence (MLIF) - LIBS combination for REE isotopes analysis. The mostly sensitive are the bands at 633.3 (1,1 transition, System I) and at 481.66 (1,2 transition, System I). Under the pumping at the 446.26 nm region (0,1 transition, System I) two strongest individual lines appear at 463.33

and 446.48 nm (pumping at 446.32 and 446.53 nm), while three found at 481.74 and 481.92 together with 481.99 nm (pumping at 446.38 and 446.65 nm).

The experiments with individual Gd isotopes are under progress.

(LIBS-04.5) Pulsed Microwave-Assisted Laser-Induced Breakdown Spectroscopy and Laser Ablation Molecular Isotopic Spectrometry Using Microstrip Waveguides

Kelsey L. Williams¹, Steven J. Ray²; ¹*The State University of New York at Buffalo*, ²*The State University of New York at Buffalo*

Method can improve efficiency and portability of microwave-assisted LIBS and LAMIS.

Laser-induced breakdown spectroscopy (LIBS) and laser ablation molecular isotopic spectrometry (LAMIS) are optical techniques that enable the measurement of a sample's elemental and isotopic composition, respectively. In both techniques, a laser is used to ablate a small amount of sample material and form a laser-induced plasma (LIP) from which atomic and molecular vibronic emission are detected. The microwave-assisted LIBS strategy uses microwave radiation to modify the LIP conditions so as to significantly improve the signal observed in LIBS atomic emission measurements. In addition, the literature has shown that the introduction of microwaves into the LIP results in increased molecular emission, which could also improve LAMIS measurements. Here, we evaluate the efficacy of several microstrip waveguide architectures and microwave power modulation strategies aimed at improving microwave energy coupling into the LIP. Since the LIP is transient, pulsed microwave waveforms can be used to apply microwave fields at various times during the LIP lifetime. In LIBS, detection is delayed approximately 1 – 10 μ s after formation of the LIP providing optimum signal-to-background ratio (S/B). In LAMIS, however, detection is delayed further to permit atomic and ionic species to interact with atmospheric gases resulting in formation of diatomic species that are used in the LAMIS measurement. Thus, microwave power can be delayed or changed in duration based on the measurement at hand. This presentation will also evaluate different microwave microstrip antennas to be used in the experiment. Microstrip antennas and resonators are created as a copper circuit atop a thin dielectric, and are designed to focus microwave power to the location where the LIP will be formed. The antennas are simple and inexpensive to create, and can thus be tailored to the LIP to promote best energy transfer. Here, the efficacy of different antenna types, such as the half-wave split-ring resonator, will be evaluated. The optimal experimental conditions and analytical performance of microwave-assisted LIBS and LAMIS will be discussed.

22PMA04: SERS for Diagnostics and BioPharma Manufacturing

Chair: Karin Balss

Co-Chair: Courtney Morder

(PMA-04.1) Inverse Molecular Sentinel Integrated Bimetallic Nanostar Substrate for Ultrasensitive Medical Diagnostics

Aidan Canning¹, Aidan Canning¹, Hsin-neng Wang¹, Tuan Vo-Dinh², Joy Q. Li², Xinrong Chen¹; ¹*Duke University*, ²*Duke University School of Medicine*

Molecular biomarkers such as microRNA (miRNA) regulate gene expression and are emerging as promising diagnostic biomarkers in many cancers, including colorectal cancer (CRC). Sensitive detection of miRNA biomarkers in biofluids has potential to aid early diagnosis. Traditional miRNA sensing methods often use PCR methods that are time and labor-intensive and require laboratory access. Despite numerous recent advancements in miRNA sensing technologies, sensitivity and specificity of these techniques remains a challenge due to the low concentration of miRNA in biological fluids.

Nanoparticle-based technologies are increasingly used for biomarker detection. A recent platform developed by our group to detect nucleic acid targets using Surface-enhanced Raman scattering (SERS) is called “inverse Molecular Sentinel” (iMS). Here, the method of fabricating highly reproducible, densely packed monolayers of bimetallic nanostars for reliable substrate to substrate comparisons will be discussed. Further, the integration and optimization of the iMS probe onto this substrate allowed for the amplification free, ultrasensitive detection of multiple miRNA biomarkers in CRC patient plasma.

(PMA-04.2) Differentiating Physical and Infectious Viral Titer using Surface Enhanced Raman Spectroscopy

Courtney J. Morder¹, Karin M. Balss², Zac D. Schultz¹; ¹*The Ohio State University*, ²*Janssen*

Lentiviruses are commonly used in biomedical applications such as gene therapy, pharmaceuticals, and vaccine development due to their ability to deliver genetic information to reprogram cells. During production of these viruses, it is necessary to purify and characterize them to ensure their efficacy and safety for treatments. During production some viruses may not form as intended, therefore it is necessary to quantify the number of virus particles in a sample that are useful for therapy. The presence of inactive virus particles can affect the determined titer. Knowing the infectious titer, rather than physical titer of the virus, is crucial to determine dose and expectations for these applications. Current methods of determining infectious titer, such as PCR or cell phenotype-based assays, require considerable sample preparation and can take days to weeks to obtain results. Surface enhanced Raman spectroscopy (SERS) is capable of detecting differences between viruses and determining the titer of a virus. Our results show SERS can be used to detect two different lentiviruses, one containing an added gene from a virus that will not encode the gene. Additional differences in the SERS signals from empty viruses provide further information to determine infectious titer. The SERS signal of the viruses on commercial substrates was recorded using various concentrations of each virus in order to determine SERS signals characteristic of both physical and infectious titer. This study also provides

insight into which components of the virus give rise to the SERS signal, providing new insight into SERS diagnostics of viruses in general.

(PMA-04.3) Designing a COVID-19 Assay using Surface Enhanced Raman Spectroscopy (SERS)

Taylor Payne¹, Stephen Klawa², Ronit Freeman², Zac D. Schultz¹; ¹*The Ohio State University*, ²*University of North Carolina, Chapel Hill*

The ongoing pandemic has intensified the demand for a rapid, selective diagnostic assay for COVID-19. The shortcomings of currently available detection methods, such as polymerase chain reaction (PCR) and antibody tests, provide motivation to explore alternative techniques that do not sacrifice accuracy for speed. Here, to detect SARS-CoV-2 we utilize surface enhanced Raman spectroscopy (SERS), which is a quick, sensitive light scattering technique, that requires minimal sample pre-processing and yields highly specific molecular signatures. SERS capitalizes on the intense, local electric fields that surround metal nanostructures under resonant laser excitation. Analytes within the fields, typically adsorbed to the nanostructure surface, give greatly enhanced Raman signals. Nonetheless, using SERS to sense viruses presents certain challenges, namely interference from biological matrices and signal variability based on the orientation of these large species on the nanostructure surface. Fortunately, capture molecules can enhance signal reproducibility by forcing the analyte into a consistent surface orientation, and they can minimize interference by selectively targeting the analyte. Peptides can be used to bind the surface proteins of viruses and capture them on SERS surfaces so that the viruses can be identified based on their SERS signatures. In this work, we have developed SERS sensors modified with a spike-binding peptide to detect SARS-CoV-2, providing a basis for the design of a SERS-based assay for SARS-CoV-2 and other possible future viruses.

(PMA-04.4) Low-Cost Sensors for the Identification and Quantification of Disease Biomarkers, Viral RNA, and Drugs Of Abuse

Laura Fabris¹, Hao Wang¹, Kaleigh Ryan², Ajita Nair², Zhaolin Xue³, Kholud Dardir², Manjari Bhamidipati²; ¹*Rutgers, the State University of New Jersey*, ²*Rutgers University*, ³*University of Massachusetts Amherst*

The integration of biosensors into various industries can transform the ability to monitor personal and public health, food safety, and the environment. Nanostructured sensors, in particular, have pushed detection limits down to femtomolar and even attomolar concentrations by utilizing diverse sensing modalities. In particular, high sensitivity and specificity have been realized using surface enhanced Raman spectroscopy (SERS), which has been proven very useful in biomarker analysis. Fluorescence-based detection often accompanies SERS; the two modalities complement each other quite seamlessly, and have now started to be leveraged simultaneously, thus pushing the boundaries of sensitivity, selectivity, and multiplexing even further. However, often these sensing platforms have the drawbacks of high production costs, limited shelf life, and complex implementation, which have hindered their widespread applicability. Our research has therefore been focusing on designing and implementing sensing, and importantly, diagnostic platforms, with reduced costs and increased applicability, that however retain sensitivity and selectivity, and can be

implemented in multiplex. In my talk, I will report on our results focusing in particular on the detection of communicable and non-communicable diseases, on the low-cost forensic analysis of opioids carried out with portable Raman equipment, and on the development of streamlined approaches for substrate characterization and data analysis that can increase SERS quantification power without internal standards. I will discuss how gold nanostars can be leveraged to design switchable SERS/fluorescent probes for the detection of influenza A viral particles in buffer and individual intact cells with high selectivity, how SERS-based biosensors can be used for phenotype characterization in individual cancerous cells, and how these devices allow to efficiently stratify prostate cancer patients. Finally, I will discuss how we leveraged a low-cost portable Raman module to identify fentanyl in urine with LODs of 5 ng/mL and to detect it when laced in other drugs of abuse.

(PMA-04.5) Indirect Surface-Enhanced Raman Spectroscopic Detection of Biomarkers Associated with Polycystic Ovarian Syndrome

Avery Wood¹, Bhavya Sharma¹; ¹*University of Tennessee, Knoxville*

PCOS diagnosis is complex; this work offers a novel sensor for potentially simpler diagnosis.

Polycystic ovarian syndrome (PCOS) is an endocrine disorder affecting one in ten women and is the leading cause of infertility in women. Commonly, PCOS is diagnosed in women when fertility complications arise. PCOS has a plethora of potential lifelong health complications, including hirsutism, infertility, obesity, type 2 diabetes, and depression. Current diagnosis of PCOS depends on the presence of the following criteria with two being present for adults and three for adolescents: hyperandrogenism, ovulatory dysfunction, and/or polycystic ovaries. These criteria can only be determined through invasive physical examinations and extensive bloodwork. To date, there is no analytical, minimally-invasive method for early diagnosis which would allow for early implementation of metabolic or pharmaceutical treatments to minimize the symptoms and long-term health impacts of PCOS. Through the use of surface-enhanced Raman spectroscopy (SERS)-based sensors, biomarkers associated with PCOS can be monitored in order to potentially lead to early diagnosis. SERS combines the signal enhancement from the electric field surrounding metal nanoparticles with the non-destructive, specific, and rapid nature of normal Raman spectroscopy (RS). Previously, we have developed an on-site immunoassay using antibody conjugated gold nanoparticles for the indirect SERS detection of Protein A down to the low femtomolar concentrations. Protein A is found in the cell wall of *Staphylococcus aureus* which can cause a plethora of diseases from food poisoning to skin, bone, or joint infections. That work established a scaffold for indirect SERS detection; this work describes the development of SERS-based sensors for the early diagnosis of PCOS through detection of biomarkers in blood serum.

22RAM04: SERS 3

Chair: Zac Schultz
Co-Chair: Royston Goodacre
Co-Chair: Sian Sloan-Dennison

(RAM-04.1) Advancing SERS Biosensors for Diagnostic Applications

Pietro Strobbia¹; ¹*University of Cincinnati*

The recent pandemic has revealed the critical need for accurate and widespread POC-testing for infectious diseases. While viral infections can be detected rapidly with colorimetric lateral flow assays (LFA), they do not provide accurate nor quantitative results and necessitate confirmatory negative tests. Conversely, confirmatory tests use the current gold standard for molecular diagnostics, reverse transcriptase polymerase chain reaction (RT-PCR), to detect viral RNA, which remains expensive and, in many cases, only available in large hospitals and health centers. Without the widespread of a cost-effective and accurate POC diagnostics for field testing, we will remain vulnerable to the spread of viral infections. Surface-enhanced Raman scattering (SERS) sensors are ideally suited to fill this gap. SERS permits to detect simultaneously many Raman peaks associate to one or multiple targets, increasing the assay accuracy and multiplexed detection. These sensors can also be designed to be homogenous, which we define as detecting the target without any extra step (reagentless and no-wash sensors). The sensitivity of SERS sensors that is often considered their limit has been recently increased by coupling the SERS sensing mechanisms with DNzyme ideas, such as catalytic hairpin assembly (CHA) and hairpin chain reaction (HCR). However, these sensors currently do not have homogenous sensing mechanisms and are very complex to design, due to the multiple strand-to-strand interaction at play. These issues have hindered their translation to POC diagnostics. We develop a recycling sensing mechanism based on a catalytic toehold exchange to amplify the sensitivity of homogenous SERS sensors. We optimized and elucidated the thermodynamic properties of the sensing mechanism to achieve high amplification. We then coupled our sensor with an automated sensor design system to obtain optimized sensor design for new targets and challenged our design system to detecting emerging viral threats.

(RAM-04.2) Towards semiconductor substrates in SERS: applications in biosensing

Kristen Dellinger¹, Samuel Adesoye; ¹*North Carolina Agricultural and Technical State University*

Surface-enhanced Raman scattering (SERS) is a phenomenon that results in the amplification of Raman signals via interactions with nanostructured substrates. SERS is understood to be a product of two mechanisms: electromagnetic and chemical. The electromagnetic mechanism is attributed to plasmon excitation in metal nanostructures functioning as a substrate, while the chemical mechanism is broadly attributed to a group of processes associated with the transfer of electrons between a molecule and substrate. While plasmonic materials have formed the bulk of research in SERS, semiconductors have

recently been proposed as materials that may benefit from the chemical mechanism. Indeed, they exhibit several properties that could be advantageous in SERS work, particularly in biosensing applications, given their excellent physical properties, unique chemistries, and tunable interactions with light. Zinc oxide (ZnO), in particular, has a wide bandgap, high binding energy, large surface-to-volume ratio, bandgap tunability, and low cost. In addition, it is widely available, highly reproducible in fabrication, and is generally considered to be biocompatible. However, its application is limited due to a low SERS enhancement. In this context, we endeavored to improve the enhancement of zinc oxide through substitutional doping, which introduces defects that may improve the charge transfer process between the ZnO and target analyte. Previously, various dopants such as Mg, Co, and Ga have been used to introduce defects into ZnO. We selected Mg to be a suitable candidate for doping ZnO due to its high solubility in ZnO lattice and its comparable radius to Zn^{2+} which could prevent lattice distortion. In this experiment, the bandgap shift resulting from doping ZnO with Mg was ascertained from the perspective of SERS applications, while its charge transfer effect and SERS enhancement efficiency on molecule was studied. ZnO's potential adoption as a SERS substrate for biomolecules will be elucidated via a cytotoxicity assay.

(RAM-04.3) Pushing the Limits of Chemical Detection at Depths with Spatially-Offset Raman Spectroscopy

Bhavya Sharma¹; ¹*University of Tennessee, Knoxville*

Detection of Raman signals at increased depth with advances in spatially-offset Raman spectroscopy.

Spatially-offset Raman spectroscopy holds great promise for detection of molecular signatures at depths through multilayered samples without having to drill holes or remove the surface layer. When SORS is combined with surface-enhanced Raman spectroscopy, termed SESORS, this combination allows for detection of ultralow concentrations molecules, while simultaneously improving detection of Raman scattering at increased depths. Challenges for detection of signals at depth are more pronounced when considering biosensing in biofluids and tissue, particularly *in vivo*, or in other turbid media. We will discuss advances in nanoparticle functionalization, along with instrumental changes, for improved detection of Raman scattering with SESORS. SESORS shows great promise for chemical sensing and imaging at tissue depths and time scales that are relevant for a variety of fields, including biomedical imaging.

(RAM-04.4) Plasmonic nanotags for the detection and treatment of glioblastomas

Samantha M. McCabe¹, Matthew E. Berry¹, Gregory Wallace¹, Neil C. Shand², Marie Boyd¹, Duncan Graham¹, Karen Faulds¹; ¹*The University of Strathclyde*, ²*The Defence Science and Technology Laboratory (DSTL)*

Nanotags were developed for detecting and imaging glioblastoma through tissue using SESORS

In the UK, there are over 12,000 new patients diagnosed with brain cancer each year. Along with having a poor quality of life, at less than 13%, the 5-year survival rate is very low. These unfortunate outcomes are often the result of the extreme difficulty in distinguishing between cancerous and non-cancerous tissues using conventional imaging techniques. It is imperative to improve patient survival and reduce the number of unnecessary biopsies. One particularly promising cancer imaging technique is surface enhanced spatially offset Raman spectroscopy (SESORS), which combines the sensitivity and specificity offered by surface enhanced Raman scattering (SERS) with the through barrier detection capabilities of spatially offset Raman spectroscopy (SORS). Using SESORS, it is possible to non-invasively detect subsurface spectra that originate from extremely specific bio-functional SERS active nanotags obscured by diffusely scattering objects such as bone and soft tissues.

In this work, plasmonic nanotags were developed for the through tissue imaging of glioblastoma, a particularly aggressive and high-grade brain cancer. Nanotags consisting of gold nanoparticles functionalised with Raman reporters, an optional silica shell, and subsequently conjugated with an antibody designed to bind specifically to the biomarker Tenascin-C overexpressed by U87-MG cell lines were prepared. Cells were grown in 3D multicellular tumour spheroid (MTS) models devised to replicate brain tumours. MTS models are used to mimic a tumour and represent the *in vivo* properties more closely than 2D cell cultures. The nanotags can be up taken into the MTS model and then located within the MTS model using Raman spectroscopy. The aim of this work was to validate the targeting capabilities of the nanotags in MTS models using SERS microscopy, with subsequent SESORS imaging being used to demonstrate through tissue detection of the nanotags in the MTS models. The unique combination of features demonstrated by the nanotags, integrated with the advantages offered by SESORS, create a promising platform for simultaneous non-invasive brain cancer diagnosis and treatment in the future.

(RAM-04.5) SERS on a chip: Multiplex detection of seroconversion and cross-reactivity of SARS-CoV-2 antibodies in non-hospitalised individuals

Malama Chisanga¹, Jean-Francois Masson¹; ¹*University of Montreal*

We demonstrate that microfluidics-integrated SERS unravels cross-reactivity of spike-specific antibodies against SARS-CoV-2 and VOCs.

Since the beginning of the novel coronavirus 2019 disease (COVID-19) pandemic, diagnostic testing of SARS-CoV-2 and associated humoral immunity, as well as massive vaccination campaigns have significantly slowed down the spread of COVID-19. Monitoring antibody response in individuals previously infected with SARS-CoV-2 is often the basis for tracking

and mitigating the effectiveness of humoral immunity over time. This is especially important at present when several highly contagious SARS-CoV-2 variants of concern (VOCs) are gradually emerging, raising serious concerns about the potency and longevity of effective COVID-19-specific antibodies against re-infection(s) with the circulating VOCs. Development of rapid and accurate assays that detect multiple antibody response dynamics is valuable for measuring seroprevalence and cross-reactivity of convalescent subjects against emerging VOCs. In this research, we developed microfluidics-integrated multiplexed SERS assay as a potential platform for simultaneous analysis of antibody levels, cross-reactivity and durability of antibodies against the spike protein of the native SARS-CoV-2 strain, and the Gamma (P.1) and Delta (B.1.617.2) variants within a single serum sample. Multiplex detection of antibody cross-reactivity was achieved in four spatially resolved channels of a microfluidic cell, where SERS-active nanotags (AuNP-conjugated with a Raman reporter and either IgG, IgA or IgM anti-human antibodies) reacted with anti-spike antibodies bound to the spike protein of the native, P.1 and B.1.617.2 immobilised on a gold-coated prism biosensor, prior to spectral acquisition. Binding antibody responses were screened in 24 non-hospitalised individuals who returned a PCR-confirmed SARS-CoV-2 positive test and fully recovered prior to the emergence of the P.1 and B.1.617.2. By using multiplexed SERS assay and chemometrics to measure multiple immunity profiles, we report simultaneous seroconversion and cross-protection conferred by anti-spike IgG, IgA and IgM antibodies against the VOCs with sensitivities and specificities of up to 97%. Persistence and binding affinity of the spike-specific isotypes up to 8 weeks post-infection onset will be highlighted, including the correlation between serum antibody levels and patient age. Our findings build on the current evidence guiding timely intervention of appropriate healthcare post-vaccination as key strategies for controlling the COVID-19 pandemic as the world is returning to normalcy.

22SPECIAL09: Analytical Imaging I

Chair: Max Lei Geng

(SPEC-09.1) Single-molecule fluorescence imaging of electrons and ions produced in iron corrosion

Lydia Kisley¹, Lydia Kisley¹; ¹*Case Western Reserve University*

A routine schematic in every general chemistry textbook shows individual electrons and metal ions undergoing reactions, diffusion, and dissolution at a pair of spatially-distinct electrodes — the cathode and anode — at a metal/solution interface. Yet, the correlation between the anode and cathode over both space and time has never been directly measured at the molecular scale. Physicists, electrochemists, and engineers give vastly different answers spanning orders of magnitude on the distance cathodic electrons can be detected away from anodic sites. We are developing single molecule microscopy to determine the spatial and timescales of anode and cathode formation and development are during the corrosion of metal films. We have demonstrated that redox-sensing molecules that have become fluorescent either by receiving an electron at the cathode or a metal ion at the anode

can be detected at the single molecule scale and can quantify corrosion rates. We are now pursuing super-resolution analysis of the dyes to determine the locations of both the cathode and anode at ~ 10 's nm spatial and ~ 1 's ms temporal resolutions. Aspects of optical and sample design to achieve super-resolutions will be discussed, along with our spatiotemporal findings for iron, and future prospects for expanding our technique to metal alloys.

(SPEC-09.2) Imaging Molecular Transport in Nanoporous Silica at Microsecond Time Resolution

Max Lei Geng¹, Hong Bok Lee¹, Ana Rodriguez, Madelyn Daley; ¹*University of Iowa*

The functional properties of nanoporous materials are controlled by the molecular transport processes occurring at microsecond time scale. The diffusion of solute molecules through the pores, the interfacial adsorption of molecules at the pore wall, and the strengths of these interactions determine the efficiency of chemical separations and rates of drug delivery in the applications of nanoporous particles.

In this talk, we present single-particle imaging of these transport properties in nanoporous silica with microsecond time resolution in kinetics. The images are constructed from molecular events and imaging fluorescence correlation spectroscopy in the confocal geometry. The diffusion rates and adsorption kinetics show a significant heterogeneity between particles and within a single silica particle. Solute molecules thus interact with individual particles with different affinities, resulting in a broadened distribution when a collection of particles are used in a given application such as chemical separations. The adsorption events reveal multiple adsorption sites at the wall surface of nanopores and suggest a complex mechanism of band broadening in chemical separations.

(SPEC-09.3) IR Spectroscopy Beyond the Diffraction Limit at Submicron and Nanoscale Spatial Resolutions via Photothermal Techniques

Curtis Marcott¹; ¹*Light Light Solutions*

Fourier transform infrared (FT-IR) instruments produce excellent spectra and have become easy to use has enabled this powerful capability to be used by many nonexperts in their research and for industrial problem solving.

However, three major limitations have prevented its adoption in many applications:

1. The spatial resolution is limited by diffraction physics to around 5 micrometers.
2. Thin (or diluted) samples are needed in order to minimize IR band saturation.
3. Reflection measurements off non-metallic samples typically produce weak signals with distorted spectral line shapes, unless they are performed using attenuated total reflectance (ATR) accessories which require contact with sample.

These limitations have led researchers and analysts to use alternative techniques, such as near-IR and Raman spectroscopy, to chemically characterize their samples because these approaches can be applied in reflection or scattering geometries with minimal sample preparation.

A new approach that uses the photothermal infrared (PTIR) response of the sample is discussed which overcomes the limitations listed above. The tip of an atomic force microscope (AFM), or, alternately, a visible laser is used to sense the photothermal response when a tunable IR pump laser wavenumber is absorbed by molecular vibrations in the sample. The resulting spatial resolution is thus determined by the diffraction limited spot size of the AFM tip (~10 nm) or visible laser diffraction-limited spot size (around 500 nm) independent of the IR wavenumber. In addition, this visible optical response is measured in a reflection geometry, resulting in IR spectra that match those acquired in transmission mode, even for thick samples.

Recent advances in photothermal AFM-IR and optical-PTIR spectroscopy will be discussed. Several example applications in the polymer and life sciences will be presented.

(SPEC-09.4) Raman Imaging Grasped the Molecular Changes During the Cell Differentiation of Human Induced Pluripotent Stem Cells into Erythropoietin-Producing Cells

Mika Ishigaki¹, Mika Ishigaki¹, Hirofumi Hitomi, Yukihiro Ozaki², Akira Nishiyama;
¹*Shimane University*, ²*Kwansei Gakuin University*

We investigated the process of human induced pluripotent stem cells (iPSCs) into erythropoietin (EPO)-producing cells using Raman spectroscopy and imaging. The fixed cells at four stages of cell differentiation (Phase I, II, III, and IV) were analyzed by partial least square (PLS) regression model developed by Raman imaging data, and the concentration variations of intracellular molecular compositions with the cell differentiation were made clear: the band intensity at 708 and 672 cm^{-1} due to the C-S-C stretching vibrational modes, the concentrations of unsaturated fatty acids with low grade of unsaturation, and the concentrations of glycoproteins. The dynamic changes of these molecular compositions during the course of cell differentiation were successfully visualized by Raman imaging. The results showed application possibilities of Raman spectroscopy and imaging to monitor the cell differentiation and discriminate the cell monitoring method for regenerative medicine using stem cells and a new physiological therapy for renal anemia using EPO produced by differentiating iPSCs.

(SPEC-09.5) Hierarchical Chemical Patterning and Imaging of Surfaces from sub-10-nm to Macroscopic Scales

Shelley A. Claridge¹, Shelley A. Claridge¹; ¹*Purdue University*

Many materials applications require interfaces with hierarchical embedded chemical functionality at scales from e.g. regenerative medicine, energy conversion) requires extending ordering up to orders of magnitude larger scales, and to amorphous and/or flexible substrates. Here, we discuss our approach to imaging molecular ordering and impacts of the interface chemistry across length scales. Additionally, we have found that it is possible to transfer these nanometer-scale chemical patterns to amorphous, elastomeric surfaces including silicon-based and hydrogel materials, which enables new applications, but requires new imaging modalities.

References: Shi *ACS Nano* **2021**. Lang *ACS Nano* **2021**. Davis *ACS Nano* **2021**. Shi *Angew Chem* **2021**. Porter *Chem* **2019**. Villarreal *JACS* **2017**. Bang *JACS* **2016**.

22SPSJ01: Near-Infrared Spectroscopy; Spectral Analysis, Imaging

Chair: Yukihiro Ozaki

(SPSJ-01.1) Development of a Monitoring Method for Peptide Synthesis with Different Amino Acid Sequences Using Near-infrared Spectroscopy

Mika Ishigaki¹, Atsushi Ito, Risa Hara, Shun-ichi Miyazaki, Kodai Murayama, Sana Tsuji, Miho Inomata, Keisuke Yoshikiyo¹, Tatsuyuki Yamamoto¹, Yukihiro Ozaki²; ¹*Shimane University*, ²*Kwansei Gakuin University*

Special peptide drugs are expected to be one of the next-generation breakthrough pharmaceuticals, and a microflow reactor is a possible method for their synthesis. In the microflow reactor, raw materials are flowed to the columns step by step, and the objected compound is obtained without isolation and purifying the intermediates. Thus, the technology has been paid attentions as a very powerful tool for process analytical technology (PAT). To establish an industrial production line with a microflow reactor, a monitoring method for chemical reactions is needed.

We aimed to develop a monitoring method for peptide synthesis with different amino acid sequences using near-infrared (NIR) spectroscopy. The detailed analysis of the NIR spectra of eight different amino acid aqueous solutions (glycine, alanine, serine, glutamine, lysine, phenylalanine, tyrosine, and proline) and tripeptide organic solutions that were composed of the eight amino acids showed the different spectral patterns in the 5000-4500 cm⁻¹ region depending on the amino acid species and amino acid sequences. In the spectra of tripeptide solutions, the NIR band due to the combinations of N-H stretching and amide II/III modes and those derived from the first overtones of amide II and amide I appeared in this wavenumber region. The quantitative evaluation of the tripeptide concentration changes that were composed of two different amino acids, glycine and proline, in the course of peptide synthesis was performed using partial least square (PLS) regression analysis. The calibration and validation results with high determination coefficients ($R^2 \geq 0.99$) were successfully obtained based on the amino acid sequences. The results showed the usefulness of NIR spectroscopy as a PAT tool for synthesizing peptides in a micro flow reactor.

(SPSJ-01.2) Understanding How Near-infrared Quality Estimation Models for Agricultural Products Work with the aid of Metabolomics

Akifumi Ikehata¹; ¹*National Agriculture and Food Research Organization (NARO)*

Near-infrared (NIR) spectroscopy is known its widely used applications to foods and agricultural products. In particular, the nondestructive measurement of sugar content of fruits is a successful example of NIR spectroscopy. However, the mechanism of the estimation model, is not necessarily understood because of the lack of information on which absorption is assigned to what vibration of what molecule. We can gain a reasonable understanding of NIR with ¹H-NMR metabolomics because it requires only a small step of pre-processing and, like NIR, it observes the hydrogen terminals of the molecule. Well

assigned peaks of high resolution $^1\text{H-NMR}$ spectra are well suited for interpretation of NIR estimation model.

VIS-NIR spectra (500-1000 nm) of intact apples and peaches were measured by a portable spectrometer (Kubota, K-BA100R) equipped with an interactance probe. Extracted juice from the flesh was diluted with deuterium phosphate buffer to set the pH to 7.0 and measured by an NMR instrument (Bruker, AVANCE 500 MHz).

First, an estimation model of sugar content was obtained from NIR spectra by PLS regression. The goal is to explain the structure of the regression vector. The given NIR and $^1\text{H-NMR}$ spectra were analyzed by heterospectroscopy (SHY) method, which compares correlation coefficients at all combinations of variable channels i.e. wavelength and chemical shift. The heatmap of SHY showed us obvious positive or negative correlations between $^1\text{H-NMR}$ signals of various sugars and the primary components at some specific NIR wavelengths. The known $^1\text{H-NMR}$ peaks confirm that fructose, sucrose, etc. are contributing them. The averaged SHY maps in the direction of chemical shift showed a pattern similar to that of the regression vector, suggesting that the variation in the NIR spectra mainly reflects the soluble solids content, which is close to the definition of Brix. On the other hand, for peaches, not only sugars related to sweetness, but also contributions from components related to ripening were detected. Specifically, it was suggested that the variation in galacturonic acid and methanol produced during the hydrolysis of pectin were also utilized for the NIR estimation.

(SPSJ-01.3) New Avenues in Quantum Chemical Simulations of NIR Spectra – from Polymers to Aqueous Matrix and Interpretation of Instrumental Difference of Miniaturized Spectrometers

Krzysztof B. Bec¹, Justyna Grabska¹, Christian W. Huck¹; ¹*University of Innsbruck*

Quantum mechanical calculations are routinely used as a major support in mid-infrared (MIR) and Raman spectroscopy. In contrast, practical limitations for long time formed a barrier to developing a similar synergy between NIR spectroscopy and computational chemistry. Recent advances in theoretical methods enabled accurate simulations of NIR spectra of molecules reaching the size of long-chain fatty acids and functional fragments of polymer chains approximated by the molecular models that capture the structural motif of the polymer. These advances provide new horizons in NIR spectroscopy on multiple levels, as the spectra calculated *ab initio* provide substantial improvement in our understanding of the overtones and combination bands. In sharp contrast to MIR and Raman spectra, the spectral lineshape in NIR region is decisively more complex, as the consequence of much higher number of overlapping bands. Theoretical NIR lineshape improves the comprehension of the spectral information and delivers direct support to basic research using NIR spectroscopy as well as applications.

Accurately simulated NIR spectra provide insights into molecular structure, interactions and dynamics. In addition to detailed NIR band assignments, this information also forms innovative support in applications. The access to rich and detail molecular footprint is essential for fundamental research and is very useful in routine analysis by NIR

spectroscopy and chemometrics. Examples include interpretation of the difference in the performance observed between different miniaturized NIR spectrometers when analyzing the constituents featuring particular molecular structure. Selectivity of a given sensor to specific chemical structures may be assessed in detail. Further, chemical interpretation of the chemometric models is permitted, associating vibrational bands with meaningful variables. On the other hand, matrix effects in NIR spectra may be better understood. These new elements integrated into NIR spectroscopy framework enable a knowledge-based design of the analysis with comprehension of the processed chemical information.

(SPSJ-01.4) **Spectrometers in Wonderland: Shrinking, Shrinking, Shrinking**

Richard Crocombe¹, Richard Crocombe¹; ¹*Crocombe Spectroscopic Consulting, LLC*

This presentation will give a brief overview of the major portable techniques (optical - NIR, MIR, Raman; MS - HPMS, GC-MS, IMS; elemental - XRF & LIBS; and emerging miniaturized techniques like NMR). The above are all 'conventional' spectroscopic techniques, reduced to a rugged portable format, and with self-contained data systems. They provide specific, actionable, information to their non-scientist operators working with them outside the laboratory - in the field - and these instruments have well-defined value propositions.

A recent development is the availability of very low cost (< \$100) multispectral sensors operating in the visible and near-infrared regions. This low cost enables them to be embedded into consumer products, for instance, smart 'white goods' appliances, personal care and fitness products, and even 'wearables'. These devices, and related consumer products, will be described.

In the future, miniature and portable spectrometers will be ubiquitous: outside the laboratory, and in your home and pocket.

(SPSJ-01.5) **Use Of Handheld FT-NIR Sensors To Rapidly Quantify Cannabinoids of Hemp, in situ.**

Cameron M. Jordan¹, Siyu Yao¹, Luis E. E. Rodriguez-Saona¹, M. Monica Giusti¹, Gonzalo Miyagusuku-Cruzado¹, Christopher Ball¹; ¹*The Ohio State University*

The ability to quantify cannabinoids with a nondestructive and rapid analytical method

Hemp is a crop that has agricultural, economic, and pharmaceutical potential yet is still being researched. Hemp is known to produce over 100 phytocannabinoids, including cannabidiol (CBD) and Δ^9 -tetrahydrocannabinol (Δ^9 -THC). Hemp is a plant that must contain less than 0.3% THC w/w, per the 2018 Farm Bill. Current analytical methods of High-Performance Liquid Chromatography- tandem mass spectrometry (HPLC-MS/MS), which is a selective and sensitive method, but is cost inefficient, time-consuming, and complex analysis. Fourier Transform Near Infrared (FT-NIR) is a non-destructive, non-invasive method with the

potential to be added to inline production settings. The analysis of FT-NIR is almost instantaneous compared to HPLC-MS/MS with a fraction of the cost.

Hemp samples were scanned using a handheld FT-NIR scanner. Cannabinoids were extracted from hemp inflorescence and analyzed by HPLC-MS/MS. The two data matrices were correlated by Partial Least Squares Regression (PLSR) and a prediction model was generated. The prediction model allowed for the differentiation between drug-type (THC >0.3%) and fiber-type (THC < 0.3%) hemp by the content of THCA (.27-.80%) and THC (.021-.056%), and the ability to quantify 4 different cannabinoids in a single measurement, including CBDA (7.7 - 20.7%). The prediction is done with a small standard error of cross-validation and with a correlation coefficient of cross-validation of $R_{cv} > 0.95$. This experimentation shows the use of a small handheld scanner to provide a faster and cheaper analysis of a very heavily regulated crop with relatively no standardized methodology of analysis. This technology will benefit hemp growers and analysts of hemp material greatly.

22ATOM04: Traditional and Atmospheric Glow Discharge Sources

Chair: Gerardo Gamez

(ATOM-04.1) The Solution-Cathode Glow Discharge: Novel Approaches and Applications

Steven J. Ray¹, Nicholas Hazel¹, Chelsey Albaladejo; ¹*The State University of New York at Buffalo*

The Solution Cathode Glow Discharge (SCGD) is a low-power, atmospheric-pressure, ambient-atmosphere microplasma that is proving to be a proficient excitation source for atomic emission spectrometry (AES). The SCGD is an atmospheric pressure glow discharge sustained directly atop a liquid sample solution, where the plasma is responsible for both sampling and exciting the material for quantitation by AES. The analytical figures of merit of SCGD-AES experiments often compete with established, conventional approaches (e.g. ICP or AAS) despite the fact that it is a very simple, small, low-cost instrument. However, the SCGD approach still suffers from several shortcomings, including disappointing limits of detection for selected elements, an incomplete understanding of the plasma/liquid interface and how it influences analytical response, and lack of a standardized SCGD geometry designed specifically for AES. Here, we discuss the latest approaches to address these limitations. In some cases, elemental coverage is improved and limits of detection (LOD) decreased by using special modes of operation. For example, arsenic and germanium LODs can be greatly improved by use of in-situ hydride generation strategies. In cases where atomic emission is weak, we have also explored the use of molecular emission from selected metal-oxides as a means of quantitation by emission spectroscopy. The use of magnetic fields to exert control over the plasma/liquid interface region will also be discussed. Here, time-varying magnetic fields will be used to control the plasma motion with the aim of controlling the introduction of liquid material into the plasma. Finally, development of a novel slit-shaped SCGD discharge structure will

be examined. The narrow cathode shape creates a sheet-like plasma, which shows improved sensitivity and lower LODs as compared to conventional designs. The operating principles of the SCGD will also be reviewed here, including experimental operating parameters, plasma conditions, analytical performance, matrix interferences, and examples of applications.

(ATOM-04.2) Glow Discharge Optical Emission Spectroscopy with Array Detectors
Arne Bengtson¹, David Bengtson; ¹*Swerim AB*

The first commercial Glow Discharge Optical Emission Spectroscopy (GD-OES) to use array detectors was introduced in year 2000. It was a compact tabletop instrument for a limited number of applications, but it paved the way for further development. Used in low-end instruments only at first, combined array and PMT high-end GD-OES instruments soon appeared on the market. Since 2000 the array detectors (CCD, CMOS) have been greatly improved, very much thanks to the widespread use of digital cameras and smartphones. State-of-the-art array detectors can now match PMT's both in terms of quantum efficiency and dynamic range. The readout rate, important for Compositional Depth Profiling (CDP), is also more than adequate. Today, high-end GD-OES instruments with all solid-state array detectors are available.

The major advantage of array detectors is of course the full spectral coverage, doing away with the need to configure each instrument with pre-determined spectral lines for the intended applications. However, there are several added capabilities of array detectors that will be presented and discussed in this talk. 1) Line selection beyond available spectral tables. Even today, the best lines for certain elements/applications have not been fully investigated. With array detectors, each user has the possibility to explore new possibilities. 2) Improved background subtraction, particularly emission from molecular species. This is crucial for accurate CDP of very thin films and/or organic coatings. 3) More accurate quantification using multiple lines from one element. This possibility remains a challenge not yet realized, and it is linked to current development of Machine Learning techniques.

Miniature spectrometers with array detectors are ideally matched to a different type of source known as Solution Cathode Glow Discharge (SCGD), which operates at atmospheric pressure and normally in air. There are several varieties of these devices, and it is a very active field of R&D. The combination of air and water-based solutions results in very intense molecular emission, yet elemental analysis at trace levels has been demonstrated. Examples of spectra and analytical results using SCGD sources will be presented.

(ATOM-04.3) Glow Discharge Spectrometry: State of the Art and Future Directions
Jorge Pisonero¹, Cristina Gonzalez-Gago¹, Nerea Bordel¹; ¹*University of Oviedo*

Glow discharges coupled to mass spectrometers (GD-MS) are known to provide a great analytical potential for direct solid analysis, in terms of sensitivity and/or in-depth resolution. Moreover, they have experienced important developments and new commercial instruments with improved performance have been produced.

Nevertheless, we considered that a critical vision of the capabilities and limitations of GD-MS, paying particular attention to GD-TOFMS and SFMS, is required. In this work, the analytical figures of merit of GDMS are described and evaluated in the context of other complementary spectroscopy techniques for direct solid analysis. Moreover, the potential of GD-TOFMS for depth profile analysis of ultra-thin layers is discussed together with some desired upgrades to further improved its analytical capabilities. Different representative samples are employed in this evaluation.

References:

J. Pisonero, J. Fandino, J.H. Nordlien, S. Richter, J. Pfeifer, C.D. Quarles, J. Gonzalez, N. Jakubowski, N. Bordel. Improving the analytical performance of pulsed-GD-SFMS for multi-elemental depth profile analysis of heat-treated Zn coatings on extruded aluminium. *Journal of Analytical Atomic Spectrometry*, 34, 2252-2260, 2019.

C. González Gago, J. Pisonero, R. Sandín, F. Fuertes, N. Bordel. Analytical potential of RF-PGD-TOFMS for depth profiling of oxidized thin film composite membranes. *Journal of Analytical Atomic Spectrometry*, 31, 288-296, 2016.

(ATOM-04.4) Nanoparticle Characterization via Glow Discharge Optical Emission Spectroscopy Elemental Mapping

Gerardo Gamez¹, Kevin Finch¹; ¹*Texas Tech University*

Engineered nanoparticles' (NP) unique properties have promoted their increasingly extensive use in many fields, for example, therapeutics and diagnostics, catalysis, energy conversion and storage, disinfection, etc. Thus, NP characterization techniques are necessary not only for ensuring the desired engineered properties of interest, but also to monitor the growing NP proliferation in the environment, and to understand the interaction of NP with biological systems. While there are many NP characterization techniques in the arsenal, it is widely recognized that alternative techniques are needed to overcome the current limitations, in particular with respect to low sample throughput.

Glow discharge optical emission spectroscopy (GDOES) features many advantages, including fast direct solid sampling, wide dynamic range quantitation, and superb depth profiling resolution down to the nanoscale. In addition, over the last few years GDOES has shown great potential for surface elemental mapping, when the GD is operated in pulsed-power mode and at higher pressures, with the added advantage of obtaining high-pixel density maps with at least three orders-of-magnitude higher throughput compared to typical techniques.

Here, the latest efforts into harnessing such advantages toward the characterization of NP dried suspension residues will be presented. We will show how optimized operating conditions lead to limits of detection that are within the requirements for studying single-cell NP uptake. In addition, it will be demonstrated how lateral elemental mapping combined with depth profiling capabilities allow NP size measurements down to a few nm. Finally, the potential for core-shell dimension characterization will be discussed.

(ATOM-04.5) Halogen Determinations using a Liquid Sampling-Atmospheric Pressure Glow Discharge Microplasma Ion Source Coupled to a Commercial Mass Spectrometer

Cameron J. Stouffer¹, R. Kenneth Marcus¹; ¹*Clemson University*

LS-APGD is a versatile ionization source, with the present effort demonstrating direct determinations of halogens

Though halogens are among the most reactive chemical species, identification of these species via mass spectrometry (MS) is a challenge due to the limited ionization capabilities of traditional ionization sources i.e., electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and inductively coupled plasma (ICP). Halogens possess high (>1000 kJ/mol) ionization energies, which these ionization sources are not able to overcome. While a natural thought, ICP approaches are not able to detect halogen ions in a negative-ion mode, making it impossible to detect the halogens. Also, being highly electronegative species, halogens form acidic vapors when they react with hydrogen in the air, causing damage to ambient ionization sources. An ionization source capable of transitioning these non-volatile substances into the gas phase for detection by MS is needed. For this aim, a liquid sampling-atmospheric pressure glow discharge (LS-APGD) has been recently developed, acting as a combined atomic and molecular (CAM) ionization source, and offering unique advantages in the detection of the halogens. The LS-APGD ionization source has the potential to ionize polar, nonpolar, and elemental cohorts, which, when coupled with MS, provides an alternative for the detection of many challenging species. For example, a number of perchlorinated and perfluorinated aromatic hydrocarbons have successfully yielded intact molecular ions. The LS-APGD was coupled with a TSQ Quantum Access Max triple quad mass spectrometer, operated in negative and positive ion modes, to directly measure fluoride, chloride, and bromide anions, and their adducts in the cationic mode, from the respective sodium and potassium halogen salts. The capability of the LS-APGD source to ionize a diverse group of species holds great promise for the detection and analysis of halogen-containing solutions in the future. A high flow rate (500 mL min⁻¹) of He permitted the isolated halogen anions in negative ion mode. In comparison, lower flow rates (200 mL min⁻¹) resulted in the halogens being associated with a number of readily identifiable adducts when operated in positive ion mode. Use of the LS-APGD as the ionization source for halogen-containing sample analysis provides heretofore unrealized capabilities not currently provided by traditional molecular and elemental ionization sources.

22AWD02: Spectroscopy Magazine's Emerging Leader in Molecular Spectroscopy Award Symposium Honoring Lu Wei

Chair: Lu Wei

(AWD-02.1) **Pre-organization & Evolution of Enzyme Active Sites using the Vibrational Stark Effect**

Steven Boxer¹; ¹*Stanford University*

Local electric fields in complex systems like proteins can be determined using the vibrational Stark effect (VSE). A direct proportionality has been established in earlier work between the activation free energy and electric field projected onto the bond undergoing charge reorganization at the active site an enzyme from which we obtain the electrostatic contribution to catalysis. In all cases, mutations made the field and catalytic

rate smaller, begging the question whether larger fields and corresponding larger rates can be created either by design or by evolution? Using the hydride transfer enzyme liver alcohol dehydrogenase, we show that mutations and metal replacements at the active site can produce both larger fields and faster rates, extending and strengthening the concept of electrostatic catalysis. By using an aldehyde inhibitor, we can measure

projections both on the carbonyl C=O bond and on the C-H (with H replaced by D) at the same carbon. This 2-directional probe can be studied both in simple solvents and at the active site of the enzyme. We find that while the fields depend strongly on solvent, the ratio of the fields projected on C=O and C-D is approximately constant over a wide range of solvent polarities. By contrast, the ratio of the field projections at the active site of LADH is substantially different, consistent with the idea that the protein creates a unique pre-organized electrostatic environment. Implications of both observations for enzyme design will be discussed.

(AWD-02.2) **Needle in a Haystack: Chasing Nanoparticles by SRS Microscopy**

Wei Min¹; ¹*Columbia University*

Many areas of science and technology deal with nanoparticles. The spatial distribution of nanoparticles inside tissues dictates the function. Yet it is technically challenging to locate nanoparticles inside vast volume of tissues. We will show how SRS microscopy can help in this important task. Several exmaples will be given.

(AWD-02.3) **Mid-infrared Photothermal Microscopy: Theory, Instrumentation, Applications**

Ji-Xin Cheng¹; ¹*Boston University*

Recently developed mid-infrared photothermal (MIP) microscopy (Review, Science Advances, 2021, 7: eabg1559) not only overcomes the diffraction limit in direct IR imaging, but also circumvents the limitations in AFM-IR. In MIP microscopy, a visible beam probes the thermal effects induced by an intensity-modulated infrared beam. MIP signals are measured in scanner manner or in wide-field manner through an interferometric scattering microscope, a phase microscope, or an interferometric objective. In wide-field MIP microscope, focal area matching of the IR beam with the visible beam and camera-based paralleled detection offer a high imaging speed. Meanwhile, thermal confinement after nanosecond IR pulse excitation offers sub-micron spatial resolution. MIP microscopy has found important applications in life science and materials science.

(AWD-02.4) **Raman Microscopy: A New Imaging Modality that Opens up Analytical Biology**

Katsumasa Fujita¹; ¹*Osaka University*

Recent advances in Raman microscopy have opened the way to access information at the molecular level within living cells. By detecting molecular vibrations, it is possible to analyze the chemical composition of living organisms and their states, providing information that could not be obtained using conventional methods. Thus, Raman microscopy provides new tools and approaches to represent and visualize the biological environment in new ways. Raman microscopy has been used to separate cell types, states, and activities. It also paved the way for visualization of small molecules to which conventional labelling methods are not applicable. Newly developed Raman probes can visualize multiple targets simultaneously, enabling ultra-fast profiling with high-content information. These techniques enable the identification of complex biological activities and states to understand biological systems and functions, opening new avenues for analytical biology.

(AWD-02.5) **Raman Image-activated Cell Sorting**

Keisuke Goda¹; ¹*The University of Tokyo*

I introduce Raman image-activated cell sorting (RIACS), a recently developed technology that performs real-time coherent Raman image-based sorting of single live cells with a throughput of up to ~100 cells per second. Specifically, the fast image acquisition of the RIACS is enabled by multicolor stimulated Raman scattering (SRS) imaging based on a pulse-pair-resolved wavelength-switchable laser. To show the broad utility of the RIACS, I show its application to diverse cell types and sizes. The technology is highly versatile and holds promise for numerous applications that are previously difficult or undesirable with fluorescence-based technologies.

22CHEM01: A New Stream of Intelligent Measurements and Data Science Part 2

Chair: Tamiki Komatsuzaki

Co-Chair: Thomas Bocklitz

(CHEM-01.1) **On-the-fly Raman microscopy with Guaranteeing Accuracy using Reinforcement Learning I: Theory**

Tamiki Komatsuzaki¹; ¹*Hokkaido University*

We present our recent study combined multi-arm Bandits algorithm in reinforcement learning with spontaneous Raman measurements for the acceleration of the measurements by designing and generating optimal illumination pattern “on the fly” during the measurements while keeping the accuracy of the diagnosis. Here accurate diagnosis means that a user can determine an allowance error rate δ *a priori* to ensure that the diagnosis can be accurately accomplished with probability greater than $(1-\delta)\times 100\%$. We present our algorithm and our simulation studies using Raman images in the diagnosis of follicular thyroid carcinoma, and show that this protocol can accelerate more than a few tens times in speedy and accurate diagnoses faster than the standard line-scanned Raman measurement

that requires the full detailed scanning over all pixels. The on-the-fly Raman image microscopy is the first Raman microscope design to accelerate measurements by combining one of reinforcement machine learning techniques, multi-armed bandit algorithm utilized in the Monte Carlo tree search in alpha-GO. Given a descriptor based on Raman signals to quantify the degree of the predefined quantity to be evaluated, e.g., the degree of cancers, anomaly or defects of materials, the on-the-fly Raman image microscopy evaluates the upper and lower confidence bounds in addition to the sample average of that quantity based on finite point/line illuminations, and then the bandit algorithm feedbacks the desired illumination pattern to accelerate the detection of the anomaly, during the measurement to the microscope. The embodiment of the corresponding microscope using a spatial light modulator will be presented separately by collaborator Prof. Katsumasa Fujita at the BMI session: A New Stream of Intelligent Measurements and Data Science 1.

(CHEM-01.2) Deep Learning Applied to Nonlinear Spectroscopy and Microscopy for System Control, Data Processing and Feature Extraction

Dario Polli¹, Arianna Bresci¹, Federico Vernuccio¹, Chiara Ceconello¹, Francesco Manetti¹, Renzo Vanna², Subir Das¹, Giulio Cerullo¹, Dario Polli¹, Salvatore Sorrentino; ¹*Politecnico di Milano*, ²*CNR-Institute for Photonics and Nanotechnologies (IFN-CNR)*

Machine Learning (ML) and deep learning (DL) are powerful Artificial Intelligence (AI) tools to boost scientific research in a broad range of fields, thanks to their capability of approximating non-linear transfer functions and solving complex tasks, leveraging their data-driven nature. In the last years, AI and photonics are developing an increasing and promising two-way synergy: on the one hand, AI approaches can control a number of complex linear and non-linear photonics processes; on the other hand, photonics can pave the way for a new class of accelerated computational paradigms in AI. In this presentation, I will present our recent results in this field: (1) The removal of cross-phase modulation artifacts from pump–probe spectroscopy measurements with high accuracy and within a short time, to extract ultrafast electronic dynamics of material systems. (2) The removal of non-resonant background from broadband coherent anti-Stokes Raman scattering (CARS) spectra, thus enabling the high-speed retrieval of the full vibrational spectrum of molecules and solids for label-free nonlinear imaging of chemical bonds. (3) Spectral denoising, to enhance the signal-to-noise ratio in CARS spectroscopy/microscopy, to extract the maximum amount of information from the measured data hypercubes with a very large number of voxels.

(CHEM-01.3) Measurement Informatics and Its Application in Science

Takashi Washio¹; ¹*ISIR, Osaka University*

Measurement technologies to acquire information for scientific analyses, industrial productions and social services play core roles in our IoT society. Most of the advanced technologies aims the measurements on various objects beyond their past limits on accuracy, resolution, sensitivity and robustness of their outcomes, while the objects often have significantly small/large size, distance, quantity, complex structure and special features with large fluctuations and noises in both time and space. To achieve their sufficient

measurement performances, they use advanced physical and chemical processes for the measurement in highly elaborate approaches. Moreover, these technologies estimate their objectives from their corresponding patterns reflected in the highly complex outcomes by the computational analyses of their measurement data. In many cases, the information on the objects must be estimated by applying highly advanced information processing and appropriate prior information.

The recent progress of machine learning, pattern recognition, signal processing and statistics is expected to provide effective measures for achieving these superior measurements. The studies do not remain the application of the state-of-the-art current machine learning techniques to the measurement, and some theoretical extensions of their basic machine learning principles are strongly needed to adapt the principles for handling the processing of the complex and noisy outcomes of the sensing devices and instruments. However, the worldwide studies still remain in a preliminary stage in spite of the importance of this research field in both scientific and industrial aspects.

Based on these considerations, a project "[Intelligent Measurement Analysis] Development and application of intelligent measurement-analysis methods through coalition between measurement technologies and informatics" has started in 2016 under the Japanese governmental umbrella program named Core Research for Evolutional Science and Technology (CREST). In this project, a methodological framework of mathematical statistics and machine learning named "Measurement Informatics" has been studied to address the aforementioned issues. In this talk, we describe this methodological framework and explain how effectively it provides many breakthroughs in the advanced measurements for science and industry. Some concrete example studies on the breakthroughs provided by the Measurement Informatics will be demonstrated.

(CHEM-01.4) Reconstruction of Purified Optical Data from Measurements Using Deep Learning

Rola Houhou¹, Thomas W. Bocklitz², Jürgen Popp², Michael Schmitt¹, Tobias Meyer-Zedler², Parijat Barman, Elsie Quansah, Orlando Guntinas-Lichius, Franziska Hoffmann;
¹*Friedrich-Schiller University*, ²*Leibniz Institute of Photonics Technology*

Reconstruction of purified optical data from measurements using deep learning

Rola Houhou, Parijat Barman, Elsie Quansah, Tobias Meyer-Zedler, Michael Schmitt, Franziska Hoffmann, Orlando Guntinas-Lichius, Juergen Popp, Thomas Bocklitz

In biophotonics, the fast acquisition of optical data, e.g., images or spectra, compromises its quality by introducing a higher noise level, artifacts, and background contributions. Therefore, computational, mathematical and statistical methods are implemented to reconstruct a purified version of the measured data, which is of higher quality. For example, the maximum entropy method and the Kramers-Kronig relation can be used to extract the Raman-like spectrum from the coherent anti-Stokes Raman scattering (CARS) spectrum, or phase retrieval methods by means of GS (Gerchberg-Saxton) can be used to improve the

data quality. However, these computational methods are time-consuming and require a priori knowledge or additional post-processing of the extracted data.

In this contribution, two cases will be presented where we used deep learning as an alternative solution to standard correction methods. The first case discusses the non-resonant background correction that directly extracts the Raman-like spectra from CARS measurements. Here, the application of deep learning via the long short-term memory network (LSTM) for the first time is discussed¹. The second showcase concerns multimodal image denoising that includes the CARS, TPEF (two-photon excited fluorescence), and SHG (second-harmonic generation) modalities. Our approach focuses on applying different deep learning methods and comparing them to a standard phase retrieval algorithm. We showed in both cases that deep learning could fulfill the need for automated and fast extraction of a purified version of the measured data, which allows the usage of biophotonic techniques in time-critical environments like clinical diagnostics.

References

¹Houhou et al., Deep learning as phase retrieval tool for CARS spectra, *Opt. Express* 28, 21002-21024 (2020)

Acknowledgment

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(CHEM-01.5) **Transforming the Food Industry with Hyperspectral Imaging**

Andrea Weeks¹; ¹*P&P Optica*

Detecting hard-to-find foreign materials and assessing quality characteristics at the same time

Recent advances in spectroscopy and computing mean that many new applications are being developed for hyperspectral imaging. In the food processing industry, it can be used to improve both food safety and food quality in-line and in real time. P&P Optica (PPO) has combined hyperspectral imaging with artificial intelligence and machine learning to provide a unique solution for the detection of foreign objects like plastic, rubber, and bone in food plants. PPO also uses this combination of technologies to offer a consistent approach to product grading, composition measurement, detection of woody breast myopathy and much more.

In this session, Andrea Weeks, R&D Project and Experimentation Lead at P&P Optica, will share what PPO has learned by applying hyperspectral imaging in this new way, and what she sees coming next for both the technology and the industry.

22CTP/EARLY04: SAS Organized Session: Navigating Challenges to Achieve Success as an Early Career Spectroscopist, Part 2

Chair: Benjamin Manard

(CTP-04.1) Recent Applications of Laser-Induced Breakdown Spectroscopy at Oak Ridge National Laboratory

Hunter B. Andrews¹; ¹*Oak Ridge National Laboratory*

Oak Ridge National Laboratory (ORNL) is a multidisciplinary laboratory encompassing research that ranges from chemistry and additive manufacturing through to biological and nuclear sciences. Laser-induced breakdown spectroscopy (LIBS) is proving a useful analytical tool for many new research thrusts. LIBS is performed by focusing a high-energy laser pulse onto a sample surface where large energy density results in ablation followed by rapid thermal breakdown and formation of a plasma plume. As this plasma de-excites, unique electronic transitions occur, emitting photons at characteristic emissions. The collected spectrum provides an elemental fingerprint of the sample. At ORNL, this technique is currently being developed as a real-time monitoring sensor for the off-gas streams of advanced nuclear reactors, to measure and map elemental distributions in plant samples, measure fundamental radiative transfer properties, quantify trace-level impurities, and examine material interaction. A brief overview of these studies will be presented along with a discussion of future areas for LIBS involvement.

(CTP-04.2) Use of Molecular Emission by LIBS for Fluoride Imaging in Epidemiology

Mauro Martinez¹, Mauro Martinez¹, Manish Arora¹, Christine Austine¹; ¹*Icahn School of Medicine at Mount Sinai*

Exposure to toxic elements is an important aspect of the exposome, the study of the totality of environmental exposures over a lifetime. Measuring element distributions in tissues that grow incrementally, such as teeth, hair, and finger and toenails are used to reconstruct environmental exposure histories in large epidemiological studies to identify critical windows associated with disruptions to health and development. Fluoride has been associated with neuro and renal toxicity but studies are hampered by the lack of a reliable biomarker to measure fluoride exposure over time. Teeth are an excellent matrix to study fluoride exposure as fluoride has a high affinity with calcium and their incremental growth can be used to measure the timing of exposure. We developed a new method to quantify fluoride in teeth using CaF molecular emission by laser induced breakdown spectroscopy (LIBS). We optimized LIBS parameters using our doped ceramic standard material to quantify and measure the fluoride distribution in teeth. The calibration curve for the Green band in 530 nm region show a R^2 of 0.972 and the lower fluoride LOD, $18 \mu\text{g}\cdot\text{g}^{-1}$. We now present results from the validation of this method in an animal model and application to a small human study. We measured fluoride in teeth from rats exposed to fluoride at varying levels and a control group. We then compared results to that obtained by ion selective electrode analysis. In this way, tooth fluoride between the three doses of exposure was significantly different ($p < 0.05$). To demonstrate application within human samples, we mapped fluoride concentration in teeth shed by children living in areas with fluoridated exposure by food and beverage. This method can be used to reconstruct a history of early

life fluoride exposure through quantitative mapping of fluoride in human teeth, and applied in large epidemiological studies to identify critical windows of fluoride exposure.

(CTP-04.3) From Planets to Plasmas: The Career Journey of a Geochemist

Alicia Cruz-Uribe¹; ¹*University of Maine*

Dr. Alicia Cruz-Uribe is a geochemist at the University of Maine, where she runs the MAGIC Laboratory and is the Edward Sturgis Grew Associate Professors of Petrology and Mineralogy. Inspired by a love of rocks and the natural world, Cruz-Uribe pursued a degree in Earth Sciences at Dartmouth College before moving on to an M.S. in Geology at Northern Arizona University. During her M.S. studies she received her first experience with ICP-MS while undertaking Lu-Hf and Sm-Nd analyses by multicollector ICP-MS at Washington State University. After two years of bulk chemistry and chromatographic separation, Cruz-Uribe traveled to Stanford University to use the SHRIMP-RG ion microprobe for trace element analyses, and has been a fan of in situ geochemistry ever since. As a Ph.D. student at Penn State, she spent two summers at the University of Mainz in Germany, where she focused her work on trace element and isotope characterization in geologic materials by laser ablation ICP-MS. She continued her analytical work as a postdoc at the Woods Hole Oceanographic Institution, where she performed high pressure and temperature melting experiments and investigated volatile and trace element chemistry in subduction zones (large tectonic plate collisions). She is particularly interested in large scale geochemical cycling of volatiles and redox-sensitive elements at subduction zones, and routinely uses a combination of SEM, EPMA, LA-ICP-MS, and SIMS analyses.

In her role as the head of the MAGIC Lab, Dr. Cruz-Uribe has had the opportunity to branch out far beyond the rocks that are found deep in the Earth, and maintains an active research program that includes geochronology and high-speed trace element imaging of everything from volcanoes to biominerals. Dr. Cruz-Uribe is an active member of the council for the International Association of Geoanalysts, and is an associate editor for the journal *Geostandards and Geoanalytical Research*.

(CTP-04.4) Days of our Lives- Elemental Analysis at a Consumer Products Company

Jennifer L. L. Morgan¹; ¹*Procter & Gamble*

Elemental analysis tools (AA, XRF, ICP-OES and ICP-MS) are commonly used for quality control purposes in the manufacturing environment. These tools are used frequently to measure raw materials, impurities, and verify finished product quality. Not as well-known is the use of elemental analysis tools in R&D to help develop superior consumer products, from shampoo to toothpaste to laundry detergent. Elemental methodologies provide mechanistic understanding of how product technologies work to deliver consumer benefits. Products are often made with metal-based active ingredients or excipients, analytical tools that evaluate the deposition and retention of these ingredients ultimately help project teams evaluate new technologies that improve delivery of these actives. Similarly, elemental analysis can be employed to understand a product's impact on the removal of metals, and this knowledge leads to improved product performance for health and/or cleaning benefits. The creation of models using elemental markers can aid in

the identification of lead technologies, guide formulation and process development, provide insight on product performance, and support evaluation of product safety. As technologies move from the upstream to downstream pipeline, elemental analysis is utilized to provide support of clinical and consumer studies that are used to support go-to-market decisions and enable claims and credentialing activities. This presentation will illustrate some real-life challenges and solutions from an industry perspective, where elemental analysis is applied in a fit-for-purpose manner, from quick troubleshooting and crisis response to work that requires rigorous method development and validation.

(CTP-04.5) **Panel & Open Discussion**

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22FORENS03: Forensic Analysis in the Lab and at the Crime Scene

Chair: Igor Lednev

Co-Chair: Marisia Fiklet

(FORENS-03.1) **National Institute of Justice: Opportunities for Novel Spectroscopic and Analytical Techniques Applied to Forensic Problems**

Frances Scott¹, Frances Scott¹, Gregory Dutton¹; ¹*National Institute of Justice*

The National Institute of Justice (NIJ) — the research agency of the U.S. Department of Justice (DOJ) — is a leading federal funder of research and development in the forensic sciences. NIJ maintains an external grant funding program that spans a broad range from fundamental research with the potential for application to forensic science, to the development of prototype devices, to the evaluation of novel instruments and methods. Strengthening the forensic sciences through R&D helps ensure that the true perpetrators of crime are identified and convicted, increasing public safety and promoting the fair administration of justice for all Americans.

Forensic science is a collection of applied disciplines that draws from all branches of science. Nevertheless, practicing forensic scientists most often tend to be concerned with the detection, collection, separation, and analysis of chemical and biological samples. Due to the unique circumstances of forensic evidence, there is an ongoing need for these analyses to be done on ever smaller, degraded, or mixed samples. At the same time, increased backlogs in operational forensic laboratories create pressure to increase the speed and decrease the cost of analysis. Balancing this is the need to ensure that these methods are rigorously validated and defensible in a courtroom context. These needs drive NIJ's continuing R&D investments in analytical chemistry and applied spectroscopy for forensic application.

An overview of NIJ's R&D portfolio will be presented, highlighting relevant examples in Trace Evidence (fibers, glass, paint, geological, etc.); Seized Drugs and Toxicology; and

Forensic Biology. The scope and growth of NIJ's R&D portfolio will be discussed, including measures of program impact and examples of notable projects. NIJ anticipates continued interest in advancing the practice of forensic science through analytical chemistry research. In this effort, NIJ strives to engage the research community to bring novel perspectives to solving forensic problems. Information on the funding cycle and anticipated funding opportunities will be presented.

(FORENS-03.2) Expert Algorithm for Substance Identification (EASI) Applied to the Mass Spectra of Structurally Similar Fentanyl Analogs

Glen P. Jackson¹, J. Tyler Davidson, Alexandra Adeoye, Samantha Mehnert, Emily Ruiz, Jacob King; ¹*West Virginia University*

This presentation describes a novel algorithm for the identification of compounds from their mass spectra. The algorithm is based on fundamental principles of statistical mechanics, uses common statistical tools in its approach and, most importantly, can provide reliable measures of uncertainty in drug identifications.

The Expert Algorithm for Substance Identification (EASI) is divided into three steps; 1) developing a spectral database of more than 50,000 spectra of more than 70 fentanyl analogs from 9 different laboratories, 2) building linear models to enable spectral predictions, and 3) using measures of similarity or dissimilarity to make binary classification decisions about substance identifications and to assess the accuracy of the identifications.

To conduct general linear modeling, we used the abundance of each fragment ion of a compound as a dependent variable and the remaining 19 abundances as possible covariates, although only 5-10 covariates are typically required to explain most of the variance in each dependent ion. Each drug therefore uses 20 linear equations to make 20 predictions, the accuracy of which can be assessed using any desired metric.

To conduct binary classification, we used measures of similarity (e.g., dot product) or dissimilarity (e.g., Euclidian distance or mean absolute residual) to compare the 20 measured abundances in a spectrum to 20 predicted abundances based on the assumption of a certain identity. We then compared the measures of similarity or dissimilarity for the thousands of spectra using different thresholds for decision-making. The effectiveness of each model for correctly identifying drugs is assessed through receiver operating characteristic (ROC) curves.

When the thresholds for nine challenging fentalogs are set to not permit any false positive identifications, the false negative error rates for EASI are typically 12% whereas conventional algorithms average closer to 30%. EASI is therefore more successful at resolving difficult-to-distinguish isomers like o-, m-, and p-methylfuranylfentanyl. EASI is extendable to any substance and any fragmentation technique in mass spectrometry through which replicate spectra of standards can be acquired.

(FORENS-03.3) Forensic Analysis of Saliva Stains on Absorbing and Non-Absorbing Surfaces by ATR-FTIR Spectroscopy

Entesar Alhetlani¹, Dalal Al-Sharji¹, Mohamed O. Amin¹, Igor K. Lednev²; ¹*Kuwait University*, ²*University at Albany, State University of New York*

Forensic analysis of body fluid traces has received great attention in recent years. Vibrational spectroscopy demonstrated a great potential for forensic applications due to the minimum sample volume requirement and nondestructive nature. Saliva stains found on different surfaces can be of significance in homicides, sexual assaults and many other criminal cases. Thus, in this study, traces of saliva from a single donor deposited on absorbing and non-absorbing surfaces to mimic forensic evidence recovered from a crime scene were analyzed using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). Saliva spectroscopic signature was successfully obtained on all analyzed surfaces showing major bands related to amides I (C=O stretching) and amides II (N-H bending), at 1639 and 1544 cm^{-1} , respectively. At the same time, minor spectral differences were noticeable for saliva stains on absorbing surfaces such as the disappearance of the thiocyanates band at 2054 cm^{-1} . Furthermore, the stability of deposited stains was investigated by collecting ATR-FTIR spectra 1, 2, 3 and 4 weeks post deposition. Interestingly, the obtained spectra for the absorbing surfaces exhibited no spectral change with the time since deposition (TSD), whereas a noticeable decrease in the saliva signal intensity was evident in the case of non-absorbing surfaces. This preliminary study aims to provide forensic practitioners with insights on the stability of dried saliva stains on various surfaces up to 4 weeks since deposition.

(FORENS-03.4) Detection of Postmortem Changes in Liver Samples using Infrared Spectroscopy

Anna Wójtowicz¹, Agata Mitura¹, Renata Wietecha-Posłuszny¹; ¹*Jagiellonian University*
Changes in FTIR spectra of liver tissues were identified after different postmortem times and conditions

Monitoring and identifying postmortem changes in biological tissues is a very important task in forensics, as it can be the key to determining the time of a crime or the death of a victim. A particular challenge is the large number of internal and external factors influencing postmortem processes, such as the circumstances of death, body position, and environmental conditions. Conducting animal experiments allows full control of the factors relevant to postmortem lesions and is easier to perform than an experiment in human tissues. Today, non-

destructive methods are increasingly being used in forensics, including vibrational spectroscopy techniques such as infrared spectroscopy. In addition to its non-destructive nature, this technique enables quick and simple measurement of the sample.

The study aimed to analyze postmortem changes in rabbit liver tissues, differing in the time of collection after animal sacrifice and the temperature of body storage, using Fourier transform infrared spectroscopy (FTIR). Two FTIR techniques were used: attenuated total reflectance (ATR) and transmission imaging. Liver samples from an animal experiment were used. The subjects of the study were samples taken immediately after sacrificing the animals and 12 and 24 hours after storing the corpse at 4 and 20 ° C. For ATR-FTIR, liver samples were homogenized, dried on glass slides, scraped with a scalpel, and transferred to the ATR crystal. For the imaging procedure, tissues were cut into 7 µm thick slices and examined on CaF₂ slides. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) methods were used for statistical data analysis.

Both FTIR techniques used allowed the detection of changes in the intensity and position of the bands, mainly attributed to proteins (amides I and II), but also to sugars and amino acids, which may indicate postmortem changes in the structure and content of these compounds. The imaging results provided additional information on the distribution of components in the liver samples. The observed spectral changes and the comparison of both used IR techniques will be discussed.

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(FORENS-03.5) High Selectivity of LIBS for the analysis of OGSR

Shelby R. Khandasammy¹, Lenka Halámková², Matthieu Baudélet³, Igor K. Lednev¹;

¹University at Albany, State University of New York, ²Texas Tech University, ³University of Central Florida

LIBS analysis of organic gunshot residues shows potential for differentiation between highly specific sample types.

Firearm related evidence is of great significance to forensic researchers. The current ASTM International standard for forensic gunshot residue (GSR) analysis uses scanning electron microscopy coupled with energy dispersive x-ray spectroscopy (SEM-EDS/X) for inorganic GSR analysis. However, this standardized method has become constrained by the advent of lead-free ammunition on the market. Recently, many researchers have focused on exploring the probative value of organic gunshot residue (OGSR) evidence. The forensic value of OGSR evidence is bolstered by many factors including recoverability. In addition,

OGSR analysis has shown the potential to achieve differentiation based on ammunition brands and/or calibers. Raman spectroscopy is a vibrational spectroscopic technique which has been investigated for GSR analysis and for OGSR analysis specifically. Raman spectroscopy is a specific, simple, and rapid analysis technique. Laser-induced breakdown spectroscopy (LIBS) is a simple, robust, and rapid analytical method which requires minimal to no sample preparation. LIBS also requires a small amount of sample for analysis. GSR analysis via LIBS has been explored by researchers in the past.

In this study Raman spectroscopy and LIBS were used together in sequence in an attempt to achieve the specific identification and characterization of OGSR particles from closely related ammunition types. The main goal was to determine if this method had the potential to differentiate between various types of ammunition stemming from the same caliber, and which were produced by the same manufacturer, based on the analysis of OGSRs generated under identical firing conditions. Prior to LIBS analysis Raman spectroscopy was used to identify particles as OGSR. Subsequently, high-resolution microscopy documented the OGSR particles' morphologies. Finally, LIBS analysis of the OGSR particles was carried out. Advanced chemometric techniques were shown to allow for very successful differentiation between the OGSR samples analyzed. This project was supported by Award No. 15PNIJ-21-GG-04153-RESS (I.K.L.) and Award No. 2019-R2-CX-0035 (S.R.K.) awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.

22LIBS05: Chemometrics

Chair: Josette El Haddad

(LIBS-05.1) Interesting Features Finder (IFF): A New Tool to Better Explore Big LIBS Data Sets

Ludovic Duponchel¹, Qicheng Wu, Vincent Motto-Ros²; ¹*University of Lille*, ²*Institut Lumière Matière*

The production of global data in the world is expanding at an astonishing pace. We are all witnessing this evolution in our research and more particularly in the field of LIBS hyperspectral imaging. Remember the size of the data sets we were handling only 10 years ago! Indeed, the development of new instrumental concepts and new experimental methodologies allow us today to acquire data sets containing up to several million spectra acquired on a single sample. Thus chemometrics tools have never been more valuable for exploring such data sets. However our analytical objectives have not changed in the face of these new data structures. Indeed, we still want to propose an exhaustive exploration highlighting the presence of both major and minor compounds and even traces. The main

problem is that minor compounds and traces are often present on a small number of pixels representing a very small variance in the spectral data set. As a consequence, most chemometric algorithms using the concept of expressed variance have difficulty detecting these compounds, especially as the signal-to-noise ratio is often limited in many LIBS imaging experiments. Based on the principle that a LIBS spectrum is a point in a multidimensional space, an imaging data set is a point cloud contained in a hypervolume, its surface being called convex hull. In the context of spectroscopy, it is shown that the spectra present on this surface are potentially the purest ones in the data set explored, the spectra within the volume being potentially linear combinations of these specific spectra. So whether a spectrum corresponds to a pure major compound or a pure trace that could potentially be located on that surface. We propose in this work a new strategy called *Interesting Features Finder (IFF)* allowing to detect spectra located on the convex hull of the data set. This will allow us to extract a list of the most informative pixels from the data set whether they correspond to major compounds or traces. We will illustrate the potential of this method on libs data sets containing more than one million spectra.

(LIBS-05.2) **Transfer Learning for Improved LIBS Analytical Performance**

Erik Kepes¹, Erik Kepes¹, Jakub Vrábel, Pavel Porizka², Jozef Kaiser¹; ¹*Central European Institute of Technology, Brno University of Technology*, ²*CEITEC Brno University of Technology*

Owing to its non-linear signal response, quantitative analysis via laser-induced breakdown spectroscopy (LIBS) is generally challenging. A major source of this non-linearity is the compounding impact of various matrix effects. Consequently, reliable quantitative LIBS analysis is generally performed by constructing a regression model using spectra from matrix-matched calibration standards of known compositions. Moreover, due to the high sensitivity of LIBS to the instrumental and experimental parameters, the calibration data generally must be collected under identical conditions as those expected by the target application. Hence, even relatively small changes in the LIBS apparatus often prompt the repeated collection of the calibration dataset.

A notable example is the currently active Mars Rovers' LIBS instruments. Namely, an extensive dataset comprising spectra of 406 calibration targets was constructed for the older ChemCam's LIBS instrument. Recently, the SuperCam's LIBS instrument's calibration required the collection of a new calibration dataset, which consists of spectra of 334 calibration targets. Meanwhile, the regression models used for the SuperCam instrument could not benefit from the available data collected by the ChemCam instrument.

In this work, we took advantage of the partially overlapping calibration datasets. Namely, we trained an artificial neural network to transform the ChemCam dataset into a form compatible with the SuperCam instrument. Consequently, the data available for training the regression model for the SuperCam instrument was considerably extended. This led to the improvement of the calibration model's performance in terms of root mean squared error of prediction of the major oxide content of the testing dataset.

(LIBS-05.3) Combination of Multiple Spectroscopy Techniques - Using Random Forest Classifiers for Correlation Analysis

Elise Clave¹, Bruno Bousquet², Gilles Dromart³, Gilles Montagnac³, Olivier Beyssac⁴, Agnis Cousin⁵, Olivier Forni⁵, Roger Wiens⁶, Sylvestre Maurice⁷, Pierre Beck⁸; ¹*Université de Bordeaux*, ²*University of Bordeaux*, ³*Laboratoire de Géologie de Lyon, ENS Lyon*, ⁴*IMPMC, Paris, France*, ⁵*Institut de Recherche en Astrophysique et Planétologie*, ⁶*Purdue University*, ⁷*IRAP-CNRS*, ⁸*Institut de Planétologie et d'Astrophysique de Grenoble -- CNRS, Université Grenoble Alpes*

The SuperCam instrument aboard the Perseverance rover enables the analysis of the Martian surface with different spectroscopy techniques. Laser-induced breakdown spectroscopy (LIBS) provides information about the elemental composition of the target. The presence of specific minerals and the crystalline structure are characterized using Raman spectroscopy, as well as visible and infra-red reflectance spectroscopy (VISIR). These spectroscopy techniques are highly complementary and each of them contributes to achieving a global understanding of the geology of the rover's environment on Mars.

Different factors make the in situ acquisition and analysis of spectra of Martian targets challenging. Indeed, the ubiquitous dust, present both on the surface and in the atmosphere, the chemical and mineralogical heterogeneities, various grain sizes, as well as the distance to the analyzed targets (LIBS can be performed from 3 to 7 meters), may impact the noise, background and signal in the spectra. Some of these effects can be corrected through data processing, by not all of them.

We aim at understanding the complementarity of these techniques in a more precise way, by studying correlations. To do so, we use Random Forest classifiers, built on either LIBS, Raman, VISIR data or combinations of the three. However, we're mostly interested in understanding the models themselves, rather than in actually using them for classification. We analyze the decisions taken by the models, the variables and thresholds that they use, depending on the chosen parameters and the nature of the training set.

(LIBS-05.4) Real-time Machine Learning Based LIBS Sensors for Aerosol and Particulate matter

Prasoon K. Diwakar¹, Pramod Kulkarni², Nicholas E. Pugh¹, Margaret Thompson¹; ¹*South Dakota School of Mines*, ²*NIOSH / CDC*

Novel Machine learning tools for aerosol characterization.

The study presents application of machine learning tools in conjunction with LIBS/SIBS and other spectroscopy tools to develop a real-time, machine learning based spectroscopy sensor for aerosol and particulate matter characterization. Researchers all around the world have developed several sensors and monitors over the years to measure aerosol and particulate matter dust including coal dust, silica, engine exhaust effectively and as promptly as possible to minimize acute exposure conditions to workers, miners etc. In addition to monitoring, it also becomes critical to classify the aerosol and particulate matter

type, its chemical composition as well as the sourcing and mapping of the aerosol and particulate matter. Application of Machine learning algorithms on LIBS/SIBS data to identify, classify and predict the provenance of aerosol and particulate matter for various industrial applications will be presented and discussed.

(LIBS-05.5) Distance of Spectroscopic Data

Jakub Vrábel¹, Erik Kepes¹, Pavel Porizka², Jozef Kaiser¹; ¹*Central European Institute of Technology, Brno University of Technology*, ²*CEITEC Brno University of Technology*

Novel distance metrics for processing high-dimensional spectroscopic data by machine learning algorithms.

Machine learning (ML) techniques are essential in a wide variety of modern spectroscopic applications. The majority of ML models use some form of distance computation. In the case of supervised learning, we may need to compute the distance of unknown spectra to the labeled representatives to decide the class correspondence. Also, in unsupervised learning, e.g. reconstruction error is considered (in autoencoders), where the distance between original data input and model output is computed.

Dealing with high-dimensional and sparse data as spectra [1], a curse of dimensionality (COD) emerges, which implies many challenges for distance computation. It is a well-known [2] consequence of COD that standardly utilized Euclidean metric is behaving poorly in high-dimensional spaces.

In the present work, we study alternative metrics to measure the distance of spectroscopic data and discuss the consequences for various ML models. Additionally, we exploit properties of spectroscopic data (high-dim., sparsity, redundancy [1]) to design novel custom metrics for distance measurement. All metrics are compared to the baseline approaches in both, supervised (KNN) and unsupervised (autoencoder) tasks. The methodology is demonstrated on Laser-Induced Breakdown Spectroscopy data.

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22LIBS07: Environmental and Cultural Applications

Chair: Madhavi Martin

(LIBS-07.1) Optimizing Hand-held LIBS Instrumentation for the Analysis of Archaeological and Historical Sites and their Environment

Vincenzo Palleschi¹, Bruno Cocciaro, Olga De Pascale, Giorgio Senesi; ¹*CNR, Italy*

The study of archaeological and historical settlements in their natural environmental context is extremely important in the perspective of environmental archaeology, the discipline that aims to the reconstruction of ancient environments by archaeo- and paleo-botanical research. In that framework, it is essential to develop and use portable instrumentation for the analysis of the geological, biological, and archaeological materials of interest. Laser-Induced Breakdown Spectroscopy (LIBS) has, in principle, all the characteristics of sensitivity and efficiency for being effectively used in this kind of studies. However, the requirement of portability reflects in poorer analytical performances of hand-held LIBS instruments with respect to their laboratory equivalent. In this talk, we will discuss the procedures that can be developed to improve the treatment of the LIBS spectra acquired with hand-held instrumentation in the study of archaeological and environmental samples.

(LIBS-07.2) Toxicity Assessment of Cadmium on Model Plants, the Case of Industrial Hemp and White Mustard

Jozef Kaiser¹, Ludmila Čechová², Pavlína Modlitbová², Zdenka Kozáková², František Krčma², Andrzej Miziolek³, Pavel Porizka²; ¹*Central European Institute of Technology, Brno University of Technology*, ²*CEITEC Brno University of Technology*, ³*SpectralCannalyzer*

Laser-induced breakdown spectroscopy (LIBS) became an established technique in the toxicology of various contaminants and the elemental bioimaging of whole plant organisms (from roots to leaves) as well as the bioimaging of cross-sections (roots and needles). Thus, LIBS provides complementary information in the detection of plant contaminants, their bioaccumulation, biodistribution, and/or translocation within the model plant organisms. Determination of exact distribution of heavy metals in plants is important for an assessment of toxicity and possible health risks. Additionally, it gives us information about spatial distribution of studied elements in the sample which enables a retrospective study of pollutant migration within the plant. It is possible to map the whole plants or only chosen parts of plant samples.

In this study, *Cannabis sativa* (industrial hemp) was grown in hydroponic solution and man-made cadmium contaminated soil. The content of cadmium in soil was varied in ranges of tens of mg/kg. Plants were grown up to 3 days (hydroponic solution) and up to weeks in contaminated soil. Toxicity of cadmium in soil was assessed on the basis of macroscopic toxicological endpoints. LIBS was used to demonstrate the spatial distribution of cadmium in plants. We found out that the cadmium was preferentially accumulated in the roots. Moreover, *Sinapis alba* (white mustard) was used as a reference plant sample as typically used in toxicologic studies.

(LIBS-07.3) Recent Advances in the Use of Laser-Induced Breakdown Spectroscopy to Classify Pathogens in Clinical Specimens

Steven J. Rehse¹, Emma J. Blanchette¹, Emily Tracey¹, August Baughan¹, Grace Johnson¹;

¹*University of Windsor*

We have been investigating the use of laser-induced breakdown spectroscopy (LIBS) for the rapid detection and diagnosis of bacterial pathogens, particularly those pathogens responsible for human infections present in clinical specimens. A rapid diagnosis that could be provided at the point of care with minimal sample preparation would improve clinical response times, reduce the overuse of broad-spectrum antibiotics, improve patient outcomes, and reduce overall treatment costs. Currently we prepare bacteria samples by depositing cells on a nitrocellulose filter medium using a custom fabricated centrifugation / cone-concentration device. The resulting thin film of bacteria is ablated in our LIBS apparatus (9 ns duration 1064 nm pulses; 8 mJ/pulse at the target; argon overpressure atmosphere; spectra collected at a delay time of 2 μ s.) Bacteria are typically suspended in megohmic water prior to deposition to reduce or eliminate background contributions to the bacterial spectrum. Bacteria are also suspended in sterile (negative for the presence of bacteria) specimens of blood and urine obtained from the pathology laboratory of a local hospital to create “spiked” positive specimens. Using this sample preparation method, we have collected several hundred spectra from five species of bacteria: *Staphylococcus epidermidis*, *Escherichia coli*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*. We have also analyzed the spectra obtained from control specimens including blank nitrocellulose filters, sterile water, and the negative control specimens of blood and urine.

This presentation will describe our efforts to obtain high signal to noise bacterial spectra from the fluid specimens and will detail our efforts to optimize chemometric algorithms, specifically partial least-squares discriminant analysis (PLSDA), to reliably detect the presence of bacterial pathogens in the nominally sterile fluids. The differences obtained when testing chemometric models with single-shot spectra as compared to summed and averaged spectra will be illustrated. The use of a whole-spectrum analysis rather than a variable down-selected model with a total of 164 independent predictor variables will be discussed. Lastly our initial efforts to implement an efficient and robust artificial neural network (ANN) algorithm to improve classification accuracy will be described.

(LIBS-07.4) Quantification of Silicon in Poplar Leaves and Wood Pellets via Laser-Induced Breakdown Spectroscopy

Hunter B. Andrews¹, Ann Wymore¹, Xiaohan Yang¹, Wellington Muchero¹, Stan Martin¹, Elizabeth Herndon¹, Natalie Griffiths¹, Gerald Tuskan¹, David Weston¹, Madhavi Martin¹;

¹*Oak Ridge National Laboratory*

Laser-induced breakdown spectroscopy (LIBS) is being employed for high-throughput elemental analysis to identify candidate genes associated with Si accumulation in poplar samples. Si accumulation can have significant impacts on an ecosystem by contributing to long-term carbon

sequestration. The candidate genes identified by LIBS Si levels will be used to create transgenic poplar plants that overexpress multiple combinations of these genes for Si accumulation. LIBS is especially promising in this regard as it can provide rapid composition information with little-to-no sample preparation. LIBS involves focusing a high energy laser pulse onto a sample surface ablating a minute amount of material to form a plasma. As the plasma cools, the electronic transitions provide characteristic emissions providing an elemental fingerprint of the sample spot. In this work, LIBS has been used test pelletized poplar bark/wood samples and quantify the Si levels. Furthermore, poplar leaf samples have been mapped to examine Si distribution throughout sample.

(LIBS-07.5) Statistical Sorting of Commingled Remains Using Portable LIBS

Kristen Livingston¹, Matthieu Baudelet¹, Jonathan Bethard², Katie Zejdlik-Passalacqua³;
¹*University of Central Florida*, ²*University of South Florida*, ³*Western Carolina University*

Field-deployable LIBS technology expedites the reassociation of individuals within commingled human remains.

The commingling of human remains is a challenging situation in both forensic and archeological contexts. Skeletal elements from multiple individuals are mixed and require sorting, or reassociation, to their respective individuals. This task is often tedious if not daunting when physical and/or visual osteometric methods are employed. However, physical traits are not the only useful discriminating factor between individuals' bones. Their chemical profile can vary from person to person as well. This study assesses whether these differences in the chemical composition of skeletal remains can uniquely classify bones into respective individual groups.

Laser-induced breakdown spectroscopy (LIBS) is the analytical method of choice to obtain such chemical profiles of bones. It requires no sample preparation and quickly renders an optical emissions spectrum that is representative of sample surface composition. Additionally, LIBS is quasi-nondestructive; there is no visible indication that material has been removed from the bone's surface. Finally, LIBS technology is available in handheld, field-deployable instruments. As much of the work in anthropology and bioarcheology happens out in the field, portable instrumentation is critical for effective and efficient analysis.

This study simulates the analysis of mass graves and the reassociation of commingled remains based on the chemical information provided by the LIBS spectra of bones. A comprehensive data set is constructed by acquiring spectra across a large subset of bones, each from multiple known individual remains obtained at decomposition facilities. Statistical models are subsequently built and tested with supervised machine learning. In conjunction with data reduction techniques, discriminant analysis algorithms, such as LDA and PLS-DA, can successfully match an unclassified bone with the individual to whom it belongs. This technique shows promise as a tool that aids bioarcheologists and forensic anthropologists in the sorting of commingled human remains. The spectral analysis of bones

using portable LIBS systems has great potential to speed up the re-association of individuals within skeletal assemblages.

22PMA02: Pharmaceutical Forensics

Chair: Ravi Kalyanaraman

Co-Chair: Scott Huffman

(PMA-02.1) Bridge the Gap: Education and Training in Career Development

Dale K. Purcell¹, Dale K. Purcell¹; ¹*Chemical Microscopy, LLC*

The education and training gap is a complex issue with many contributing factors of knowledge and practical skills that requires a multifaceted response, which brings together educators, businesses, and policy. Education is gaining the theoretical knowledge and training is the inculcation of specific skills in a scientist. This presentation will introduce several “gaps” in technological knowledge of diverse instrumentation employed in pharmaceutical forensic investigations. Mentorship and apprenticeships require commitment from senior management to the frontline supervisor and the workers at the site. The key to an effective apprenticeship is the role of the experienced scientist as a teacher or coach for the new scientist. It is shown that most skill learning occurs during the “hands-on” learning sessions.

(PMA-02.2) FTIR Microscopy: big information from small samples

Mike S. Bradley¹, Mike S. Bradley¹; ¹*Thermo Fisher Scientific*

Meeting the demands of the modern analytical laboratory means responding quickly, economically, and flexibly to a high volume of samples from many sources. The pressure to deliver successful results fast increases all the time; using state-of-the-art FTIR microscopy tools can help you meet those demands.

Microspectroscopy can analyze particulates, fibers, inks, contaminated surfaces, and more, making it a workhorse tool across a wide range of industries from environmental to automotive and pharmaceuticals. Forensics and research labs need to address a broad range of sample types.

This seminar will demonstrate how the latest in microspectroscopy hardware and software helps users address analytical challenges across different sample types encountered in these diverse settings. The speaker will show how diffraction-limited IR optics combined with outstanding visual performance and powerful, 64-bit software with a completely new approach to the user interface can improve the speed and accuracy of your results and enable a wider range of users to access the information, especially when productivity is measured in microns and minutes.

(PMA-02.3) Pharmaceutical Forensics in Cell Therapy - Ensuring patient safety and product supply in autologous cell therapies through great science, collaboration, and patient mindset.

Jeremy Peters¹, Alex Iew, Ravi Kalyanaraman¹, Scott Huffman¹, Brittany Handzo¹; ¹*Bristol Myers Squibb*

Pharmaceutical Forensics is about utilizing high level analytical and scientific capabilities to solve real world problems in commercial and clinical pharmaceuticals to enable effective decision making, determine sources of issues and patient impact, and directly leads to quality outcomes. Performing this work when we have unexpected outcomes, whether small or large, ensures we understand and scientifically prove that product and patient impact, and enables trust in our decision making to outside parties. This scientific due diligence has been extensively developed and utilized in both the small molecule and biologics modalities over the years by BMS and the Forensics & Innovative Technology (FIT) group. Beginning in 2021 however, barriers were broken and effective trust and collaboration began between FIT and a new and exciting product modality, Cell Therapy. With still much the industry can better learn and understand about our cell therapy products, adopting established analytical and characterization techniques that aren't currently utilized, and thinking innovatively about exciting new technologies will be extremely important. This effective collaboration over the last year and a half has enabled decisions that impacted dozens of individual patients desperately need these therapies, and established an effective way of thinking about future ways of working and operating. This journey is still early in its infancy, but this presentation will focus on how it was created and enabled, and the analytical techniques and strategies adopted and utilized in Cell Therapy to effectively enable data driven decisions to impact our patients.

(PMA-02.4) The Patient Found What? Real Foreign Matter Complaints Received by Bristol Myers Squibb Forensics Laboratory

Brittany Handzo¹, Scott Huffman¹, Ravi Kalyanaraman¹; ¹*Bristol Myers Squibb*

To describe the pharmaceutical forensics process/analyses conducted by the BMS-FIT laboratories.

The Forensics & Innovative Technologies (FIT) team at Bristol-Myers Squibb conducts analytical testing associated with foreign/extraneous matter from manufacturing complaints, customer generated product quality complaints (PQC), and suspected counterfeit drug products. A PQC investigation is defined as any customer generated, manufacturing, or packaging complaints related to the quality and safety of a product. This includes possible failure of product specifications or dissatisfaction with the appearance, package, labeling, or components of the product. PQC complaints can be initiated from anyone in possession of a product, such as patients, doctors, or pharmacies. Typical analytical testing conducted for PQC investigations includes characterization of foreign material, authentication of drug products, or moisture testing for broken/disintegrated tablets.

PQC investigations are unique due to the non-conventional nature of the requests. Once a product is commercial and is assessable to patients, anything can happen since the product is outside BMS control. The objective of this work is to highlight the pharmaceutical forensics processes and analyses conducted by BMS-FIT, including real foreign matter complaints such as foreign objects, hairs, metallic scuffs, and more. Analytical techniques such as microscopy, spectroscopy, elemental analyses, and occasionally chromatography coupled with mass spectroscopy are used for these forensics analyses. The analytical workflow will be described, starting from sample receipt, sample analysis, and ending with investigation closure.

22RAM13: TERS

Chair: Andrew Whitley

Co-Chair: Andrea Centrone

(RAM-13.1) Sub-Diffraction Nanoscale Raman Imaging of the Interface in a 2D Semiconductor Heterostructure

J. Pierce Fix¹, J. Pierce Fix¹, Sourav Garg², Andrey Krayev³, Audrey Sulkanen⁴, Minyuan Wang⁴, Gang-Yu Liu⁴, Patrick Kung², Nicholas Borys¹, Juan M. Marmolejo-Tejada¹, Martin A. Mosquera¹; ¹Montana State University, ²University of Alabama, ³HORIBA Scientific, ⁴University of California Davis

J. Pierce Fix¹, Sourav Garg², Andrey Krayev³, Juan M. Marmolejo-Tejada⁴, Audrey Sulkanen⁵, Minyuan Wang⁵, Gang-Yu Liu⁵, Martin A. Mosquera⁴, Patrick Kung², Nicholas J. Borys¹

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Transition metal dichalcogenide (TMD) semiconductors are solids composed of single sheets of atomic layers that are weakly bonded together via the van der Waals interaction. A single layer yields a 2D atomically thin semiconductor with a direct band gap and strong light-matter interactions. This work studies the interface in a single-layer 2D MoS₂/WS₂ lateral heterostructure with a spatial resolution of 50 nm using resonant and non-resonant tip-enhanced Raman scattering (TERS) imaging and spectroscopy [1]. In conventional confocal Raman spectroscopy, a spatial resolution on this scale is not possible because of the diffraction limit. With the sub-diffraction spatial resolution and the

vibrational fingerprinting ability of Raman spectroscopy, TERS allows us to probe the composition, size, and heterogeneity of the 2D system on length scales most relevant for nanoscale optoelectronic technologies. We use TERS to reveal that the alloyed transition region varies in size from 50-600 nm within a single crystallite. TERS nanoscale imaging of the transition region allows for tracking of vibrational modes as they evolve across the $\text{MoS}_2/\text{Mo}_x\text{W}_{1-x}\text{S}_2/\text{WS}_2$ system [2]. This work demonstrates the capabilities of TERS in characterizing monolayer lateral heterostructures on the nanoscale.

[1] S. Garg, J. P. Fix *et al.*, “Nanoscale Raman Characterization of a 2D Semiconductor Lateral Heterostructure Interface”, *ACS Nano* **16**, 340 (2022).

[2] J. M. Marmolejo-Tejada, J. P. Fix *et al.*, “Theoretical analysis of the nanoscale composition, tip-enhanced Raman spectroscopy, and electronic properties of alloys in 2D MoS_2 - WS_2 heterostructures”, *J. Phys. Chem. C* (in press).

(RAM-13.2) **Symmetry-Prohibited Modes in Tip-Enhanced Raman Spectroscopy**

Andreas Ruediger¹, Mohammad Bakhtbidar, Alexandre Merlen, Azza Hadj Youssef;

¹*Institut National de la Recherche Scientifique - Énergie, Matériaux et Télécommunications*

First order Raman spectra obey the selection rule that the change of polarizability of a normal mode for very small amplitudes has to be non-zero. Non-zero implies that even small perturbations may in fact activate a mode that would otherwise be Raman-silent even though its intensity may remain relatively small. Among different intrinsic nanoscale features that provide symmetry reduction such as strain gradients around dislocations, surfaces, surface steps, and alike, we also discuss recent observations of polar Raman modes and IR-modes in otherwise Raman-inactive strontium titanate that we attribute to surface modes and the possible effects of strong fields and field gradients in the proximity of the tip. The fact that symmetry-reduced signatures from a small volume experience tremendous enhancement in tip-enhanced Raman spectroscopy (TERS) even if they stem from a small volume, designates TERS as a sensitive probe for these modes that are often a precursor for phase transitions. Recent suggestions that plasmon-enhanced spectroscopy techniques may also alter the selectivity of modes due to an additional momentum from the plasmon i.e. making first order tip-enhanced Raman spectroscopy a 3 particle process of photon-plasmon-phonon are briefly discussed.

(RAM-13.3) **Tip-enhanced (Non)Linear Hyperspectral Nano-Imaging of Molecules and Plasmons**

Chih-Feng Wang¹, Patrick El-Khoury¹; ¹*Pacific Northwest National Laboratory*

Plasmon-enhanced hyperspectral nano-imaging has attracted significant attention in recent decades because of its great spatial resolution and information context. This technique is mature for linear nano-optical processes, including tip-enhanced Raman spectroscopy (TERS) and tip-enhanced photoluminescence (TEPL). Indeed, both techniques have been amply used to characterize molecules, excitons, polaritons, and hot carriers on the nanoscale. In our multimodal hyperspectral nanoscopy (MHN) platform, we demonstrated close to 1 nm spatial resolution in Raman imaging under ambient laboratory conditions using gap-mode TERS. We used the nano-optical response to probe local optical fields at

the surfaces of plasmonic metal nanoparticles. Tip-only TEPL on the other hand was used to visualize quantum dots within 5 nm spatial resolution, and to detect defects (N-V centers) in nano-diamonds. Besides linear nano-optical studies, the nonlinear responses such as coherent anti-Stokes Raman scattering (CARS), parametric four-wave mixing (4WM) more generally, second harmonic/sum-frequency generation (SHG/SFG), and two-photon photoluminescence (2PPL) can also be detected with similar spatial resolution. Such measurements are still challenging, due to nonlinear optical cross-sections and huge background from the plasmonic probe and otherwise metals. The flexible excitation and collection channels in our MHN setup and the field enhancement at plasmonic tip-sample nanojunctions facilitate the tip-enhanced nonlinear optical (TENON) studies. We explored these nonlinear processes on the nanoscale (<2 nm). Here, excitons in two-dimensional transition metal dichalcogenides were tracked using tunable non-resonant CARS. Moreover, plasmon-driven 4MW, SHG, SFG, and 2PPL were simultaneously spatio-spectrally resolved with sub-2 nm spatial resolution, again under ambient conditions. These measurements show that local plasmonic resonances change dramatically on the few-nm length scale. The effect has very recently been explored more directly through tip-enhanced nano-extinction measurements. Our results and described setup pave the way towards a real space-time (nano-femto scale) characterization of molecules and materials.

(RAM-13.4) Horibal Bio-TERS: from 2D Materials to Cancer Cell Nanoimaging
Dmitri Voronine¹; ¹*University of South Florida*

Tip-enhanced Raman scattering of biological systems (referred to as Bio-TERS) is one of the most challenging applications of TERS due to the high complexity of biological “wet, warm and noisy” environment with weak Raman signals. Many biomolecules have common structural motifs that result in overlapping vibrational bands with a high spectral congestion. Biological fluorescence background may overwhelm the Raman signals. Flexible structure and fluid environment may lead to temporal fluctuations of localized Raman spectra. Overcoming these challenges will be rewarding in realizing a big potential of TERS for rapid, sensitive, and non-invasive detection and identification of biological systems, including DNA and protein sequencing, probing local environment at the single biomolecule level in viruses, bacteria, cancer cells and other systems. Here we discuss some key highlights using selected examples of our TERS experiments performed on a range of samples from the atomically thin 2D materials to biological cells using a state-of-the-art commercial HORIBA instrumentation. These novel experiments provide first steps towards the promising potential of Bio-TERS for sensing and imaging applications including bactericidal inactivation and cancer therapy.

(RAM-13.5) Panel & Open Discussion

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22SPECIAL04: FACSS 2021 Charles Mann Award Symposium Honoring Roy Goodacre

Chair: Royston Goodacre

(SPEC-04.1) **Mann Alive! ... Or is He**

Duncan Graham¹; ¹*The University of Strathclyde*

The presentation will be a mix of science and stories about the awardee. The Awardee has dished it out for many years and now it's time to hear about their exploits in science and beyond. The science aspects will focus on using SERS combined with lateral flow assays to provide new capability for disease diagnostics at the point of care.

(SPEC-04.2) **Is this Mann Awake?**

Karen Faulds¹, Hayleigh Kearns, Duncan Graham¹; ¹*The University of Strathclyde*

Surface enhanced Raman scattering (SERS) is an analytical technique with several advantages over competitive techniques in terms of improved sensitivity and multiplexing. We have made great progress in the development of SERS as a quantitative analytical method. Many methods exist and fluorescence spectroscopy dominates the detection technologies employed with different assay formats. Another advantage of SERS over existing detection techniques is that of the ability to multiplex which is limited when using techniques such as fluorescence. We have clearly demonstrated the ability to identify and quantify the presence of a mixture of 3 pathogenic DNA sequences in solution using data analysis techniques.

Here we demonstrate the development of new bioanalytical assays based upon SERS which have been used successfully for the detection of bacterial pathogens using modified SERS active probes. Biomolecule functionalised nanoparticles have been designed to give a specific SERS response resulting in discernible differences in the SERS which can be correlated to the presence of specific pathogens. In this presentation the simultaneous detection and quantitation of 3 pathogens within a multiplex sample will be demonstrated.

(SPEC-04.3) **COVID-19 Diagnostic and Prognostic Analysis Using Mass Spectrometry: Weighing Viruses and Consequential Metabolic Response.**

Katherine A. Hollywood¹, Kathleen Cain, Ellen Liggett, Reynard Spiess, Caitlin Walton-Doyle, Eleanor Sinclair, Andrew Pitt, Perdita Barran; ¹*University of Manchester*

COVID-19 has had an undeniably marked effect on life across the globe. In the summer of 2020 we looked into the feasibility of utilising mass spectrometry as an alternative viral detection approach to traditional RT-PCR methods. In conjunction with the UK Department of Health and Social Care and the National Health Service (NHS) and a consortium of UK-based academic laboratories we embarked on a pilot study to assess the applicability of such an approach. There were three pre-requisites for our method; firstly, it should have clinical utility, it should have ability to be widely adopted and it should compare with RT-PCR in terms of sensitivity and specificity. To this aim we embarked on a large phase 1 study where multiple academic laboratories set about to develop a targeted proteomics-based detection method from traditional oral nasopharyngeal swabs and also from saliva. Protocols were optimised but sensitivity remained a challenge. To overcome this, we incorporated a capture

method where digested peptides specific to the nucleocapsid protein bind specifically to antibodies tethered to magnetic beads. This approach was a game changer and allowed us to compete with the sensitivity limits of RT-PCR. This method has now been handed over to 12 UK clinical mass spectrometry laboratories where they seek towards method accreditation and roll out to generic population testing. This government-funded program is an outstanding real-world example of a MS test being translated from academic labs to clinical laboratories across the country. Alternatively, we are also greatly interested in the prognostic analysis of COVID effect. We are currently undertaking metabolomics, lipidomics and proteomics analysis of a large cohort of COVID patients seeking further prognostic markers present within serum and saliva. This talk will provide an insight into the challenges associated with developing a clinically robust MS detection method and also illustrate the wealth of additional prognostic information available within biological matrices.

(SPEC-04.4) Raman Optical Activity: Raman Spectroscopy for the Twisted

Ewan W. Blanch¹, Ewan W. Blanch¹; ¹*RMIT University*

Highlighting Charles Mann awardee Roy Goodacre's contributions to collaborative research into ROA and SERS.

Raman optical activity (ROA) measures a small difference in Raman scattering from chiral molecules using circularly polarized light, while SERS has matured into a widely used analytical technique because of the high levels of sensitivity that can be provided through the local electric field enhancement generated by plasmon resonances from certain nanomaterial surfaces. Both of these derivatives of conventional Raman scattering provide unique perspectives of molecular structure and have led to many revelations and exciting studies in fundamental and applied sciences. The combination of ROA with SERS through surface enhanced ROA (SEROA) has more recently been explored by several research groups, challenging the determination of outstanding PhD students (on behalf of the supervisors: sorry guys!) but also presenting new insights into the complex interactions between chiral systems, circularly polarized photons and energy. This talk presents relevant examples of our research over 20 years into ROA, SERS and SEROA, in the context of the great help and support provided by Charles Mann awardee Professor Roy Goodacre as we travelled down this twisted, but fascinating, path of discovery. In honor of Roy's many contributions, there will also be some jokes.

(SPEC-04.5) Raman-based bioprocess monitoring and control: new technologies and applications

Ian Lewis¹; ¹*Endress+Hauser*

Raman spectroscopy has been used in industrial bioprocessing for over 10 years. Raman spectroscopy is a leading analytical tool in upstream bioprocessing due to its robust, scalable, and proven technology. Raman provides deep levels of real-time process understanding and feedback control without destructive sampling. Raman is increasingly being utilized to overcome longstanding challenges faced when implementing QbD

principles. Within the past 10 years, there has been a rapid evolution of technology and applications as well as industry drivers toward increased non-spectroscopist use and application sophistication. We highlight new technologies that enable model transfer, control strategies beyond glucose, probe compatibility with micro/mini and single-use bioreactors, downstream process control, and integration to MVDA and automation platforms. These developments enable non-specialists to harness the power of Raman quickly and easily, as we move closer to the goal of turnkey PAT.

22SPECIAL10: Analytical Imaging II

Chair: Max Lei Geng

(SPEC-10.1) **Presentation Title TBD**

Jefferson Chan;

(SPEC-10.2) **"Locking On" to Single Molecules and the Extracellular Phase of Viral Infection**

Kevin D. Welsher¹; ¹*Duke University*

Single-molecule measurements have the power to uncover heterogeneity and dynamics obscured by bulk methods but are limited to surface-tethered phenomena. This talk will introduce a new active-feedback 3D microscope (3D Single-Molecule Active Real-time Tracking or 3D-SMART) that can overcome this hurdle to capture the dynamics of rapidly diffusing single molecules in solution. 3D-SMART "locks" single target fluorophores in the focal volume of an optical microscope using real-time feedback to move the sample and compensate for molecular diffusion at diffusive speeds up to 10 $\mu\text{m}^2/\text{s}$. 3D-SMART serves to "untether" single-molecule spectroscopy measurements, dramatically expanding the application scope of these measurements to solution-phase chemical processes. Critically, this technique is readily extended to dynamic biological processes, such as the earliest critical events in the viral infection process: penetration of virions through the epithelial layer. When combined with a rapid volumetric imaging method (3D Tracking and Imaging, 3D-TrIm), we show that active-feedback single-virus tracking can capture the previously unobserved early events in the interactions between single viral particles and live cells. This new method can propel single-virus tracking from simple monolayer culture towards more tissue-like conditions by tracking single virions in tightly packed epithelial cells, leading to new insights into this earliest stage of the infectious cycle.

(SPEC-10.3) **Exploiting Infrared Light-Matter Interaction to Advance Nanoscale Characterization and Nanomanipulation of Materials**

Laurene Tetard¹, Laurene Tetard¹; ¹*University of Central Florida*

Bypassing the limitations of infrared spectroscopy imposed by optical diffraction can be achieved by monitoring of behavior of a material excited by infrared light with the nanoscale tip of an atomic force microscope (AFM). The improvement in spatial resolution and sensitivity afforded by nanoscale infrared (nanoIR) spectroscopy are expected to bolster our fundamental understanding of heterogeneous materials and living systems. However,

important aspects of light-matter interactions involved in nanoIR measurements remain uncharted, giving many opportunities to advance the performance and functionalities of the approach.

In this talk, we will first present the general concept of nanoIR spectroscopy and the current state-of-the-art. Next, we will describe how multi-frequency AFM developments can be controlled to improve the sensitivity and spatial resolution of nanoIR spectroscopy and imaging. In particular, we will discuss how the nonlinear nature of the tip-sample interaction in AFM can be used to synthesize new virtual modes to carry out highly sensitive measurements. Next, we will show that it is possible to boost nanoIR signals using plasmonic substrates, which can be designed for strong enhancement of a narrow IR band or for a “broadband” enhancement of the IR fingerprint. We will highlight some measurements carried out to quantify the performance of the plasmonic substrate while taking into account the nanoscale detection scheme of the AFM. Finally, we will show how plasmonic behaviors at the tip-sample contact region can be exploited for the deterministic creation of nanoscale defects, the formation of which can be monitored with nanoIR spectroscopy. A perspective on the opportunities of these new advances will be proposed.

(SPEC-10.4) Integrated Simultaneous Chemical, Surface Potential, Mechanical Imaging at < 10 nm Spatial Resolution

Xiaoji Xu¹; ¹*Lehigh University*

A new type of integrated nano-IR and Kelvin probe force microscopy is developed.

Multimodal measurements of chemical composition, electrical properties, mechanical properties, and topography by scanning probe microscopy (SPM) deliver correlations across properties at the nanoscale, and provide clues to the structure-function relationship of materials. In the past, measurements with these modalities are operated separately with different operational modes of SPM. Not only do the sequential measurements require additional operation time and are subject to scanner/sample drift, but also different modalities of SPM have different spatial resolutions, which undermine correlative analysis. For example, the popular frequency-modulated Kelvin Probe Force Microscopy measures the surface potential with 30~50 nm spatial resolution under the ambient conditions, whereas the SPM measurements of chemical composition, mechanical properties, and topography can routinely achieve < 10 nm spatial resolution.

At the conference, we will present our invention of an integrated SPM mode that can simultaneously provide chemical, surface potential, mechanical, and topographic imaging at < 10 nm spatial resolution under ambient conditions. We name it peak force infrared-Kelvin probe force microscopy (PFIR-KPFM), as we achieved it through an integration of peak force infrared microscopy and pulsed force Kelvin probe force microscopy. In a single scan, the integrated PFIR-KPFM delivers simultaneous multimodal measurement at a comparable and high spatial resolution of < 10 nm. As a demonstration, we measured a naturally-

degraded $\text{CH}_3\text{NH}_3\text{PbBr}_3$ perovskite single crystal. AFM topography, mechanical modulus, contact potential difference (CPD), and nano-IR imaging at infrared absorption of perovskite are simultaneously acquired. We have also studied amyloid fibrils with the PFIR-KPFM. Correlations between infrared absorption and CPD indicate that secondary structures of proteins may absorb charges differently.

(SPEC-10.5) **Advancements in Mid-IR Imaging Techniques for the Study of Biological Liquid-Liquid Phase Separation**

Arnaldo Serrano¹, Claire Nelmark, Arnaldo Serrano¹; ¹*University of Notre Dame*

Liquid-liquid phase separating (LLPS) peptides and proteins have drawn a great deal of research effort over the past decade due to their relevance in many biological functions and systems, as well as their involvement in various neurodegenerative diseases. While current evidence links condensates to a large number of biological functions, the role they play in determining protein structure has proven difficult to study directly. We propose the use of mid-IR hyperspectral imaging to directly measure differences in peptide and protein structure and solvation dynamics between the condensate and bulk phases that are characteristic of LLPS. As a vibrational technique, mid-IR hyperspectral microscopy offers a label-free alternative to traditional fluorescence imaging of complex environments like condensates, with an added sensitivity to chemical structure and dynamics. However, mid-IR microscopy comes with its own set of challenges. With a diffraction limit of $\sim\lambda/2$, we lose the ability to capture fine details and structures within the sample using solely traditional imaging methods and optics. Here we describe improvements to our spectroscopic imaging techniques, including microsphere imaging, oblique and structured illumination, for use in mid-IR spectroscopy and microscopy of LLPS condensates that will aid in the development of resolution enhanced mid-IR hyperspectral imaging.

22SPR03: Biosensing with Plasmonics

Chair: Emilie Ringe

(SPR-03.1) **Improving Selectivity for Plasmonic Biosensors**

Amanda J. Haes¹, Amanda J. Haes¹; ¹*University of Iowa*

Gold nanostars exhibit size and shape tunable plasmonic properties. In this presentation, we explore how fine morphological variations influence (or not) their plasmonic properties. Implications of morphology are used simultaneously to tune the adsorption of small molecules in sensing applications using surface enhanced Raman scattering (SERS). Because molecules must interact with the metal at short distances, molecule-surface interactions are important for successful small molecule detection. Herein, the surface chemistry of Good's buffer-stabilized gold nanostars is evaluated using computational, microscopic, and spectroscopic methodologies. Ultrasensitive detection is facilitated via

weak intermolecular interactions. As a result, we expect these studies will broaden the scope of SERS and plasmonic-based assays as small molecules with weak affinity to metal nanostructures will be more readily detected.

(SPR-03.2) A Selective and Sensitive Aptamer-Based Surface Plasmon Resonance Biosensor for Serotonin Detection

Clarice E. Froehlich¹; ¹*University of Minnesota, Twin Cities*

Serotonin is a neurotransmitter with great clinical significance due to its involvement in various physiological processes within the body. For example, serotonin levels in blood and urine have been used as a clinical diagnostic tool for illnesses like cancer and diabetes. To work towards developing a quick and simple serotonin sensor, surface plasmon resonance (SPR) was used to investigate the binding interaction between serotonin and an aptamer affinity agent, and was further used as a direct sensing platform. SPR provides many advantages over other common sensor platforms, including time-resolved measurements, compatibility with many types of biomolecules, and high sensitivity. However, due to serotonin's very small size (176 Da) and positive charge at physiological conditions, low sensor responses and non-specific interactions with the negatively charged aptamer and sensor surface presented a significant issue. Through the use of streptavidin-biotin coupling for aptamer immobilization, as well as a high ionic strength buffer for charge screening, these issues were minimized, allowing for the detection of binding events. These techniques allowed for the determination of the equilibrium dissociation constant (K_d) of the interaction as 360 nM, as well as for evaluation of the selectivity of the aptamer against other small molecules present in blood such as GABA, tryptophan, and histamine, which all had insignificant effects on sensor response at normal relevant concentrations. The sensor was also found to regenerate in less than 30 seconds under the flow of buffer, allowing for reusability of a single aptamer-modified sensing surface for upwards of 50 samples.

(SPR-03.3) Theranostic Applications of Plasmonic Nanoprobcs based on Surfactant-free Caged Gold Nanostars

Aidan Canning¹, Xinrong Chen¹, Ren A. Odion¹, Tuan Vo-Dinh²; ¹*Duke University*, ²*Duke University School of Medicine*

Development of a nanoparticle platform with photothermal, in vivo tumor detection, and drug delivery applications.

Recently our laboratory has been exploring novel and unique plasmonic nanoparticles for a wide variety of nanoplasmonic applications. Our group pioneered the first surfactant-free and therefore biocompatible synthesis of gold nanostars (GNS), which act as highly efficient photothermal transducers that can be used to treat solid tumors and help trigger a long-lived immune response. Also, we have investigated the incorporation of ultrabright surface-enhanced Raman scattering (SERS) 'nanorattles' as tags for the detection of malaria from infected blood in an amplification-free nucleotide hybridization assay.

In this work, a surfactant-free synthesis was developed to combine the previously mentioned types of nanoplatfoms in order to encase GNS within a gold nanoshell, resulting in caged GNS. The sub 10-nm thick gold shell is formed between the core of the

nanoparticle and the branch tips, resulting in no significant increase in nanoparticle size. This novel nanoparticle platform retains the near IR plasmonic resonance peak desirable for in vivo photothermal therapy, while at the same time having a loadable compartment for Raman-active dyes or small molecule therapeutics. Here, we will characterize and review the theranostic applications of caged GNS including photothermal therapy of solid tumors, in vivo SERS detection for tumor detection and margining, and localized release of small molecules upon external stimulus.

(SPR-03.4) Surface Plasmon Resonance Imaging (SPRi) in Combination with Machine Learning for Microarray Analysis of Multiple Sclerosis Biomarkers in Whole Serum
Alexander S. Malinick¹, Daniel Stuart¹, Alexander S. Lambert¹, Quan Cheng¹; ¹*University of California Riverside*

Applied machine learning to SPRi to differentiate three multiple sclerosis specific antibodies in whole serum

Multiple sclerosis (MS) is the most common autoimmune disease observed in young adults and is notorious for being incredibly difficult to diagnose. Current diagnostic methods are considered unreliable and inefficient, as they typically lack the needed specificity that allows for routine monitoring of disease progression. MS is noted by the attack of the immune system to various components of the myelin sheath such as gangliosides, sulfatides, and proteins, and anti-ganglioside antibodies are considered potential biomarkers that may differentiate MS from other diseases that exhibit similar symptoms and reportedly range between 3 to 20 ng/mL in MS patient serum samples. In this work, we will describe an SPR imaging method in combination with carbohydrate microarrays for the detection of MS biomarkers of anti-GT1b, anti-GM1, and anti-GA1 antibodies in undiluted serum. A working range of 1 to 100 ng/mL was demonstrated with the limit of detection for the three investigated antibodies to be below 7 ng/mL. This performance suggests suitability of the method for the clinical assessment of antibody abnormalities in patient serum. The microarrays utilized in the presented study were coated with perfluorodecyltrichlorosilane (PFDTs) to form a self-assembled monolayer (SAM), allowing for the antigenic sensing sites of the gangliosides to be displayed in a well-organized and easy to access manner. Coupling machine learning (ML) to the carbohydrate microarray and SPRi allowed for the investigation of the observed cross reactivity between the three investigated antibodies. K-nearest neighbor (knn) and neural networks (nnet) were employed to analyze both end point data and SPRi sensorgrams for the evaluation of the binding interactions that include both kinetic and steady state components. The success of these models has the potential to solve one of the major concerns of label free detection methods which is the uncertainty of what has been captured and detected by the sensor. The presented work has the potential to be used as a platform for the swift detection and diagnosis of MS using blood samples.

(SPR-03.5) LSPR Sensing on Nanofibers and Highly Curved Objects

Jean-Francois Masson¹, Maryam Hojjat Jodaylami¹, Necka Aka, Emilie Ringe²; ¹*University of Montreal*, ²*University of Cambridge*

LSPR sensors are typically performed on flat substrates or with colloidal particles in suspension. In some cases, it can be interesting to develop spatially addressable sensors or develop plasmonically active packing materials based on micron or sub-micron substrates. These substrates are typically highly curved in the form of cylinders, cones or spheres, leading to optical responses governed by the plasmonic properties of the nanomaterials and optical effects (resonator, lensing, etc.) from the circular shape of the substrate. In this presentation, data will be presented to better understand this interplay of plasmonic and optical responses on nanofibers, on glass beads and on quartz fibers, with the aim of developing a nanosensor for proteins secreted by cells. Data on photocatalysis and on photothermal effects will also be presented.

Poster Presentations

Tuesday Poster Session - ATOM

(Tu-P02) **Analysis of Micronutrients in Fruit Juice by Inductively Coupled Plasma Optical Emission Spectroscopy**

Andrea M. Palpini¹; ¹*PerkinElmer Inc.*

Fruit juice is a popular beverage and is often preferred as an alternative to artificially flavored and carbonated drinks as it can be a valuable source of vitamins, minerals, and fiber. The nutritional content of 100% fruit juice is derived from the fruit itself and these nutrients are displayed on the bottle label. This labeling information serves an important purpose as a source of information for consumers and is legally required in North America. With the accuracy of this labeling being the responsibility of the manufacturer, it is important to have a means to quantify the content of food products, including micronutrients, for both safety and quality reasons along with regulatory label-claim requirements. Analytical testing introduces the benefits of not only testing the final product but also the raw materials used during manufacturing. Additionally, having an accurate and precise method of analysis allows for the optimization of the production process to maximize nutrient yield or production volume where appropriate.

Inductively coupled plasma optical emission spectroscopy (ICP-OES) is generally the preferred technique for the multi-elemental determination with detection capabilities suitable for micronutrients. Alternative techniques are available, such as flame atomic absorption spectroscopy however, this method of analysis is single element determination requiring the reanalysis of each sample per analyte which can be time consuming. This work focuses on the multi-elemental determination of micronutrients in a variety of commercial fruit juice products using a microwave digestion system for sample preparation with ICP-OES as the chosen technique for analyte detection.

(Tu-P03) **TotalQuant Technique - more than Semi-Quantitative Analysis**

Ewa M. Pruszkowski¹, Chady Stephan¹; ¹*PerkinElmer Inc.*

TotalQuant (TQ) is a software feature unique to the NexION ICP-MS platform for quantifying 80 elements in a sample, in a single run, by heuristic interpretation of the complete mass spectrum. Measuring the full mass range requires only a couple of minutes and the spectral calculations itself takes just a few seconds. During the TotalQuant analysis, each element is assigned a response value (cps/ppm) which is updated when a calibration is performed.

Even though TotalQuant is an ideal tool for semiquantitative analysis during the method development, it can also be used for a final material characterization. Additionally, TotalQuant could be used as a tool for fingerprinting and a fast survey scan of unknown samples before quantitative analysis.

This poster will show variable applications of this versatile technique, called – TotalQuant.

(Tu-P04) Fast, High-Resolution Full Elemental Laser Ablation Imaging using Time-Of-flight ICP-MS for Endogenous Metal Analysis and Label Identification in Biological Samples

Lukas Schlatt¹, Phil Shaw¹; ¹*Nu Instruments*

Bioimaging is a valuable tool to gain insight into the elemental composition of biological thin sections. Using the newest laser ablation techniques single bursts of data can be generated in a matter of a few milliseconds. This requires the fast detection to enable the fastest and most accurate possible recording of images. Since the analysis of as many elements as possible is typically required a TOF-ICP-MS becomes an important part of a modern LA-ICP-MS system for bioimaging. The power of the TOF-ICP-MS to record transient full mass spectra in sub-millisecond dwell times enables the full potential of the laser ablation systems creating large images at high resolutions in a matter of a few hours or even minutes. Furthermore, the full elemental detection enables the analysis of endogenous elements as well as higher mass isotopes often used as markers to further understand the metalomics and metabolomics in living organisms. Data will be presented showing the latest images of biological materials highlighting the speed, resolution and sensitive detection of many elements. Spot sizes down to one micrometre were able to be used while still being able to ablate multiple square centimetres due to the modern ablation systems and fast acquisitions times. This allows an in-depth examination of endogenous elements for detailed analysis of the metabolome of biological samples on a cellular level.

(Tu-P05) Development and Validation of a Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) Method for the Analysis of Multivitamins

Claudia Martinez Lopez¹, Todor I. I. Todorov¹; ¹*US Food and Drug Administration*

Monitoring nutritional and toxic elements present in food, dietary supplements, and cosmetics is part of the United States Food and Drug Administration's mission to protect and promote public health. The analysis of toxic elements in multivitamins typically involves nitric acid digestion followed by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) analysis. The use of acid digestion is not only time consuming, but also leads to known health and safety concerns and the generation of hazardous waste.

Laser Ablation (LA) – ICP-MS offers a quick and automated sample analysis without the need for corrosive acids and extensive sample preparation, while reducing hazardous waste and carryover contamination. Additionally, LA-ICP-MS is especially useful for products that are resistant to nitric acid digestion and require the use of hydrofluoric and/or perchloric acids. A method for the LA-ICP-MS analysis of multivitamins was previously developed by our group. We now describe the initial steps towards the validation of the analytical method by reporting recovery, limits of detection, precision, and linearity.

Multivitamin samples were quantified by LA-ICP-MS using cellulose and matrix-matched calibration standards. A set of 10 multivitamins and 3 reference materials were fortified with the elements of interest (i.e., As, B, Co, Cr, Cu, Fe, Mg, Ni, Na, Pb, S, Zn) by the addition of a cellulose multielement powder in the appropriate mass fractions. Multivitamin tablets, calibrants, and reference materials were digested using hydrofluoric/nitric acid, analyzed by solution ICP-MS, and compared to the laser ablation results. All samples were ablated using a UV excimer laser followed by ICP-MS analysis. Instrumental parameters were optimized to obtain the best signal while reducing fractionation and the variability between replicate measurements. Method limits of detection and quantitation are measured as 3 and 10 times the standard deviation of ten cellulose blank samples, respectively. Data on recovery, precision, and calibration linearity of the method are reported.

(Tu-P06) Iodine content in seaweed sold in the United States

Todor I. I. Todorov¹, Mesay M. Wolle¹, Sean D. Conklin¹; ¹*US Food and Drug Administration*

Seaweed consumption is rapidly increasing in the western world with an average growth of 7% per year in the United States. Seaweeds are rich in macro- and micronutrients but can also accumulate toxic elements if grown in contaminated waters. Thus, monitoring elemental content of seaweed intended for human consumption is important. In this study, we report the quantification of iodine and 26 other elements in 46 samples belonging to 13 different seaweed species. The samples were dried, ground to fine powder, and homogenized. Iodine was extracted using tetramethyl ammonium hydroxide and subsequently analyzed by inductively coupled plasma mass spectrometry (ICP-MS) under alkaline conditions. The iodine accumulation was highly varied between the different species, with concentrations between 15 and 3600 mg/kg. Kombu, rockweed, and oarweed had the highest levels of iodine and nori and sea lettuce had the lowest. Elemental concentrations of iodine and 26 other elements will be presented in this poster presentation. Furthermore, we will show associations between the elements and comparisons with the results of prior studies.

(Tu-P07) Wavelength Dependent Photochemistry of Styrene Azide at Cryogenic Temperatures

Dinindu P. Mendis¹, Anna Gudmundsdottir¹, Katrin Vilinsky¹, Baker Alomari¹; ¹*University of Cincinnati*

Wavelength dependent photochemistry is a way of forming distinct products from the same starting material by using different wavelength of light. In this work we studied the photochemistry of styrene azide (**1**) in solution and cryogenic matrices as a function of irradiation wavelength. Irradiation of **1** with light above 300 nm at cryogenic argon matrices and glassy mTHF matrices results in formation of azirine **2**. In contrast, irradiation of **1** with light below 300 nm in cryogenic matrices resulted in formation of azirine **2** and ketenimine **3**. Furthermore, azirine **2** reacted further with short wavelength irradiation to form ylide **4**. At ambient temperature irradiation of **1** in argon- or oxygen saturated solution gave azirine **2**. Ultra-fast transient spectroscopy with 340 nm laser made it possible to detect the singlet excited state of **1** ($I_{\max} \sim 474 \text{ nm}$, $t = 608 \text{ ps}$). We will present and discuss the mechanism for the observed photoreactivity of **1** in solutions and cryogenic matrices, and support it with DFT calculations.

(Tu-P08) **Photodynamic Behavior of 1-(2-Azidophenyl)-3,5-Dimethylpyrazole**
Janaka P. Kavikarage¹; ¹University of Cincinnati

Herein we present the photodynamic behavior of 1-(2-azidophenyl)-3,5-dimethylpyrazole (**1**) in crystals. The photochemistry of **1** in solution and the solid state has been reported previously.¹ Upon irradiation in solution it forms a singlet arynitrene that cyclizes to form 1,3-dimethylpyrazolo[1,2-a]benzotriazole, meanwhile, some of singlet arynitrenes intersystem crosses to form triplet alkylnitrene that abstracts a hydrogen from a methyl group and from 1-methylpyrazole[1,2-a]quinoxaline. We are investigating the solid-state photolysis of **1** to determine what product is formed within crystals. We are also doing solid state laser flash photolysis to identify the intermediates formed within the crystal lattice upon irradiations.

We showed with digital microscopy that irradiation of yellow needles of **1** crack both vertically and horizontally across the crystals surface, presumably due to product formation and to release of N₂ gas from the crystals lattice. We used force field calculations and SEM images to attempt to correlate the photodynamic behavior with the crystal lattice of **1**.

Reference

Albini, A.; Bettinetti, G.; Minoli, G. Chemistry of Nitrenes Generated by the Photocleavage of Both Azides and a Five-Membered Heterocycle. *J. Am. Chem. Soc.* **1991**, *113*, 6928–6934.

Tuesday Poster Session - BIM

(Tu-P09) **Mechanistic Studies Of Flavanone Synthesis Using Flow Photochemistry**

Niroodha R. Pitawela¹, Anushree Das¹, Anna Gudmundsdottir¹; ¹University of Cincinnati

Flavanones are a diverse group of secondary metabolites present in all plants. Along with the carotenoids, flavanones are responsible for the vivid colors in fruits and vegetables. Flavanones have beneficial anti-inflammatory effects and antioxidant properties, preventing diseases such as cardiovascular disease, diabetes, cancer, and cognitive diseases such as

Alzheimer's and dementia. Flavanones have been synthesized chemically using chromanones and cinnamic acid with catalytic conditions. Moreover, flavanones have been prepared by the cyclization of 2'-hydroxychalcones using acidic reagents such as sulfuric acid. Treatment of 2'-hydroxychalcones with bases also afforded flavanones.

We have shown that chalcone derivatives can be converted selectively into the corresponding flavanones using visible light. The reactivity of the chalcone is concentration sensitive. Thus, we have been using flow chemistry to ensure the photoproducts are formed selectively in high yields. The mechanism for the photoreactions was elucidated using laser flash photolysis and Density Functional Theory (DFT) calculations. The mechanism will be discussed and used to explain why the reactions are concentration sensitive.

(Tu-P10) Automated Feeding System for Normoglycemic Blood Storage

Logan D. Soule¹, Lauren Skrajewski¹, Dana Spence¹; ¹*Michigan State University*

When properly collected, separated, and stored, red blood cell (RBC) units can be utilized for transfusions for up to 6-7 weeks post collection. However, during this period, the RBCs age, undergoing irreversible physiological damages termed "storage lesions" that can affect their functionality after transfusion. We hypothesize that hyperglycemic storage conditions originating from high glucose additive solutions contribute to these storage lesions. Previous data has shown that storing RBCs in normoglycemic additive solutions over several weeks with periodic, manual glucose feeding resulted in more functional RBCs, exhibiting greater ATP release, increased deformability, and decreased sorbitol levels. However, this work did not utilize commercial blood bags, and manual glucose feeding cannot be reasonably translated to current blood banking practices. Here, we describe a first-generation automated glucose feeding system that can dispense precise volumes of a concentrated glucose solution into stored RBCs. To accomplish this, an IV bag containing 100 mM glucose was connected to a solenoid valve, which was subsequently connected to a 100-150 mL veterinarian blood collection bag containing stored human RBCs. The solenoid valve was controlled by an Arduino Uno microcontroller programmed to deliver voltage to the solenoid valve every 3 days during the 44-day storage period. The voltage-controlled opening of the valve allowed gravity to deliver a bolus of glucose solution to the stored RBCs from the above-hanging IV bag. Successful calibration of the valve enabled precise control of dispensing volume and a lower dispensing volume limit of approximately 200 μ L, with an error of less than 7% over the range of valve opening times tested. Further calibration with a 100 mM glucose solution dispensed into a normoglycemic additive solution resulted in expected glucose increases, similar to theoretical glucose increases calculated from the volume of glucose solution dispensed and the initial volume of additive solution. This calibration resulted in an error of approximately 0.5 mM across the range of volumes tested. The valve system successfully maintained physiological glucose concentrations in stored RBCs in the range of 4-6 mM for 44 days without intervention. Results suggest this technology has potential for future implementation in blood banking practices.

(Tu-P11) Development of a Catalytic Sensing Mechanism to Enhance the Sensitivity of Homogenous Surface-Enhanced Raman Sensors for Viral Genetic Targets

Steven M. Quarin¹, Amanda Macke¹, Ruxandra Dima¹, Pietro Strobbia¹; ¹*University of Cincinnati*

Viral outbreaks, such as COVID-19 and Zika, have shown the need for early, rapid, and accurate point-of-need testing, to control the spread of these diseases. Viral RNA has been used as a biomarker to surveil these diseases but is often present at extremely low concentrations (fM - aM range) at early disease stage. Currently, polymerase chain reaction (PCR) is the standard detection method for the identification of these infections due to its high sensitivity, detecting the viral diseases at early stages. However, PCR is costly, time intensive, requires the sample to be contaminant free, and specialized laboratory settings, thus limiting the turnaround of test results. Our group is developing sensors that can detect viral RNA at low concentrations for use at the point of need. These sensors are based on catalytic DNA self-assembly and surface-enhanced Raman scattering (SERS). The catalytic feature permits to recycle the target nucleic acid sequence, lowering the limit of detection for the target (increasing sensitivity). SERS permits to perform multiplexed detection of genetic targets. Additionally, the homogeneous (reagentless) design of this sensor removes the need for sample preparation and processing steps, reducing the complexity and amount of time necessary to use the sensors. Our hypothesis is that we can optimize this recycling mechanism by tuning the thermodynamic parameters (hybridization regions, hairpin loop neck) of the functional DNA strands used in the sensor to improve sensitivity and diagnostic accuracy. Understanding the role of these thermodynamic parameters will permit to translate these findings into a program to automate the design of sensors for new viral targets, and later multiplexed the detection of multiple viral targets at the same time.

(Tu-P12) Difference of Electronic Transition of Saccharides and its Monosaccharide ; Aime to the Unlabeled Analysis of Saccharide by Attenuated Total Reflection Far-UV (ATR-FUV) Spectroscopy

Ryosuke Sasaki¹, Yusuke Morisawa¹; ¹*Kindai University*

[Introduction, Purpose] Carbohydrates are important components of life, along with proteins, lipids, and nucleic acids. Glycans, in particular, are important because they play an important role in the transmission of information in life, developments of unlabeled analysis of saccharides are needed to probe their function in vivo. Saccharides that cannot be detected by conventional UV spectroscopy have allowed electronic transitions in the far-ultraviolet (FUV; 140-200 nm) regions. The electronic transitions are sensitive to molecular structures and are expected to be developed for unlabeled structural analysis of saccharides. We measured the FUV spectra of sucrose and its monosaccharide using the ATR-FUV method.

[Experimental] ATR-FUV spectra of 0.50 M~2.50 M aqueous solutions of glucose, fructose, sucrose, and thin films of sucrose were measured. In aqueous solutions, the molar absorption coefficient ϵ spectra of the sugars were obtained after performing the Kramers-Kronig transformation and subtracting the water absorption. The experimental epsilon spectra were compared with simulated spectra obtained by time-dependent density

functional theory (TD-DFT) calculations. The spectra were discussed for various water adducts to examine the effect of hydrogen bonding with water.

[Result and Discussion] In the case of the fructose mono aqua cluster, the most stable structure is the structure that has hydrogen bonding(HB) of C3-O...HOH(C3A). As an effect of the HB, electronic transitions around 160 nm for fructose monomer shift to the shorter wavelength region. Taking into account the effect of HB, experimental epsilon spectra can be assigned. The ϵ spectra of fructose and glucose were different around 150 nm. In addition, sucrose showed absorption at 160 nm in addition to the absorption of the constituent monosaccharides. No concentration dependence was observed in the respective epsilon spectra. In this presentation, we will discuss the structure of the constituent monosaccharides and their glycosidic bond by comparing them with simulated spectra.

(Tu-P13) Numerical Investigation on Microfluidic Devices to Maintain Purity and Concentration of Separated Fractions of Bioparticles

A K M Fazlul Karim Rasel¹, Sean L. Seyler¹, Mark A. Hayes¹; ¹*Arizona State University*

Characterizing biologically important materials such as exosomes, viruses, organelles, biomarkers, and proteins is the key to understanding their function. Since these particles are present in small quantities in complex mixtures of biomaterials, separating the desired analyte into a pure and concentrated form is challenging. Gradient-insulator-based dielectrophoresis (DEP) in microfluidic devices has emerged as a high-resolution separation technique in the last two decades. Polarizable particles experience the DEP force under a nonuniform electric field. The DEP separation technique exploits differences in the DEP response of particles, while the insulator-based microfluidic device's sawtooth geometry is vital to enabling high-resolution DEP-based separation. The sawtooth design has a series of increasingly narrow constrictions (“gates”) where the nonuniformity of the electric field and, therefore, the DEP force becomes large, and particles can be trapped. Previous studies have demonstrated that the sawtooth geometry successfully traps particles such as bacteria, viruses, insulin vesicles, and proteins. However, there are no reports of successfully recovering the trapped bolus of the analyte for further analysis. In particular, obtaining highly pure and concentrated analytes can later be used, for example, for characterizing samples on NMR analysis, mass spectrometry, electron microscopy, or any disease diagnostic test. Here, we propose a microfluidic device design that successfully recovers a bolus of particles. We conducted simulations based on finite element analysis on two different overall designs of sawtooth microchannels. Within those designs, side channel width and position relative to the capture zone were varied. Two methods were used to drive the particles towards the side channel outlet: electroosmotic and pressure-induced flow. This comparative study suggests devices with two inlets and one outlet per capture zone can recover the particles with higher mass transfer and maximum concentration than devices with one inlet per capture zone. This numerical investigation is the foundation for the next generation of sawtooth-based DEP devices that can transport the DEP trapped bolus in a pure and concentrated form out of the device.

(Tu-P15) Screening and Subsampling: A Successive Analysis of Nile Red Stained Microplastics Using Nanoparticle Tracking Analysis, Fluorescence (Hyperspectral) Imaging and Particle Correlated Raman Spectroscopy

Eunah Lee¹, Julie Chen Nguyen¹, Bridget O'Donnell¹, Li Yan¹, Li Yan¹; ¹*HORIBA Scientific*

A recent report on microplastics found in human blood [1] received widespread coverage by media.[2] Researchers have been actively pursuing the route of introduction and implications of microplastics on human health. While it is not yet clear how microplastics affect human health after ingestion, it is clear that only small particles may cross biological barriers (e.g. organ walls, cell membranes, etc.). Interest in small microplastics (e.g. $\leq 5 \mu\text{m}$) is increasing, down to submicron sizes ($\leq 1 \mu\text{m}$).

By nature, samples collected from environments are enormous in quantity (e.g. liters and kiloliters), containing a staggering number of microplastics. Counts would be even higher for smaller sized microplastics. It is necessary to screen and sub-sample before analysis, and a popular method is selectively staining microplastics with Nile Red stain.[3]

The analytical dilemma is the necessity of high spatial resolution and high throughput analysis. For example, using a high magnification objective lens enables analysis of small microplastics but the small field of view requires taking a large number of 'pictures' to analyze a single filter, increasing data size and analysis time.

In this paper, we propose a successive analysis method for Nile Red stained small microplastics using Nanoparticle Tracking Analysis (NTA), fluorescence (hyperspectral) imaging, and particle correlated Raman spectroscopy (PCRS). NTA can screen samples containing Nile Red stained microplastics, and measure counts and sizes of microplastics. Fluorescence (hyperspectral) imaging can screen Nile Red stained microplastics. PCRS can perform chemical identification and classify shapes of microplastics.

[1] Discovery and quantification of plastic particle pollution in human blood

Heather A.Leslie, Martin J.M.van Velzen, Sicco H.Brandsma, A. DickVethaaka, Juan J.Garcia-Vallejo, and Marja H.Lamoree

Environment International, Volume 163, May 2022, 107199

[2] <https://www.theguardian.com/environment/2022/mar/24/microplastics-found-in-human-blood-for-first-time>

[3] Rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red

Thomas Maes, Rebecca Jessop, Nikolaus Wellner, Karsten Haupt and Andrew G. Mayes
Scientific Reports, Volume 7, Article number 44501, 2017

(Tu-P16) Inertial Microfluidics for the Separation and Enrichment of Microscale Particles

Elizabeth Ruscitti¹, Stephen C. Jacobson¹; ¹*Indiana University*

The separation and enrichment of micron and submicron particles without causing excessive shear stress to biologically relevant particles are becoming increasingly important in

diagnostics. The idea of performing “liquid biopsies” by analyzing extracellular vesicles (EVs) is just one example where a gentle, yet continuous, separation method is desired. Inertial microfluidics allows for passive and continuous separation of micron and submicron particles based on their particle diameter and the principles of flow. In 1961 Segré and Silberg demonstrated that a uniform suspension of millimeter-sized particles in Poiseuille flow focused into an annulus within a straight pipe. This demonstration indicated that there were previously neglected, significant inertial effects present that led to neutrally buoyant particles migrating laterally across streamlines. In addition, secondary Dean flows can be created by the centrifugal forces imposed on parabolic flows traveling through curves or regions with an abrupt change in cross-sectional area. Consequently, secondary Dean forces combined with shear gradient and wall interaction lift forces permit inertial separations in much shorter channels. With the aid of computational fluid dynamic simulations, we are designing microchannels with non-linear geometries that combine inertial effects and diffusive transport to enrich and separate biologically relevant particles.

(Tu-P17) Vis-NIR Spectral Characterization of Joint Tissues for Arthroscopy

Amanda Spurri¹, William Querido¹, Mohammed Shahriar Arefin¹, Chetan Patil¹, Nancy Pleshko¹; ¹*Temple University*

In vivo assessment of molecular changes during repair of musculoskeletal tissues has been challenging to implement, and remains the focus of many novel imaging studies. Application of visible-near infrared (Vis-NIR) spectroscopy for monitoring of cartilage tissue during arthroscopy is a potential approach for nondestructive evaluation of tissue composition. While this methodology of tissue assessment offers the potential to provide quantitative evaluation of molecular changes during cartilage repair in a minimally invasive manner, there are challenges in implementing the technology for in vivo applications. A primary challenge in utilization of this technology is differentiating the spectral contribution of irrigation fluid (primarily water) to that from cartilage and bone molecular water. The objectives of this work are to utilize both in vitro cartilage samples and cadaver joints to evaluate the impact of external water on the tissue spectra, determine spectral features for differentiating irrigation water from tissue samples, and assess potential for future in vivo studies. During these studies, a fiberoptic probe was configured with a glass spacer extending from the probe fibers, enabling contact with the tissue samples in a water environment. Initial experiments included obtaining spectra in the vis-NIR regions from porcine nasal cartilage, both with no additional water and in a hydrated environment. Following completion of these in vitro experiments, cadaver joint studies aim to further validate the methodology for tissue evaluation during arthroscopy. Preliminary results from this study show that a probe design allowing direct sample contact limits the overall external water contribution to the sample spectra, enabling assessment of tissue composition without the resulting spectra being saturated by the irrigation water features. Additionally, analysis of second derivative spectral intensities at 7245 cm^{-1} , an absorbance arising from free water, indicates potential differentiating spectral features between cartilage water and irrigation water. Examining the spectral differences between water and cartilage during these initial studies supports both the development of the strategy

for use in future in vivo experiments and evaluation of the technology feasibility for clinical application.

(Tu-P18) Differentiation of Neurotoxic Arsenic Species in Biological Fluids Using Surface-Enhanced Raman Spectroscopy (SERS)

Paula A. Evans-Pimiento¹, Bhavya Sharma²; ¹*University of Tennessee*, ²*University of Tennessee, Knoxville*

Arsenic is a naturally occurring heavy metal element present in water, food, and soil. Released through everyday practices such as agriculture and industrial use, arsenic is rapidly emerging as an environmental pollutant. The mechanisms and effects underlying arsenic neurotoxicity are not well-understood. Studies have failed to determine the degree of neurodegeneration that occurs as a result of arsenic exposure. With exposure to arsenic, degenerative, inflammatory, neoplastic variations, cognitive deficits, changes in neurotransmitter metabolism leading to alteration of synaptic transmission, encephalopathy, and peripheral neuropathy occur. Detection methods for acute arsenic exposure do not exist, hence there is a need for the development of rapid, sensitive, and selective sensors for detection of arsenic species. Arsenic exists in the environment in two oxidative states, pentavalent (As⁵⁺, arsenate) and trivalent (As³⁺, arsenite). Arsenite is highly toxic when compared to arsenate due to its longevity in the body. Here we present the detection and differentiation of arsenic species using surface-enhanced Raman spectroscopy (SERS) at physiologically relevant ranges in artificial cerebrospinal fluid (aCSF). Combining SERS with principal components analysis, we detected and differentiated arsenite and arsenate levels in biofluid with high specificity at ultra-low concentrations. This research provided a multifaceted detection approach to lethal neurotoxic compounds that would be useful in the medical and scientific fields.

(Tu-P19) Enrichment of Green Fluorescent Proteins by Gradient Insulator-Based Dielectrophoresis

Jerry Sheu¹, Mark A. Hayes¹; ¹*Arizona State University*

Dielectrophoresis (DEP) is the phenomenon in which a dielectric particle is subject to the nonuniform electric field, and it could potentially generate a high-resolution separation of proteins in comparison to traditional methods such as size exclusion chromatography. The study of DEP force on proteins is still incomplete. Based on the classic theory proteins are too small to induce sufficient DEP force to overcome diffusion. Evolving theories suggest the dipole moment of protein is proportional to the DEP force. This work aims to inform the investigation of DEP force by using two similar proteins in regard to size, structure, pI value except for dipole moment. In this study, wild-type green fluorescent protein (wtGFP) and enhanced green fluorescent protein (eGFP) are selected, and their dipole moments are 402 D and 333 D respectively, which are estimated by Protein Dipole Moments Server.

The research of influencing proteins through DEP has been studied by alternating current DEP and direct current DEP (DC-DEP). The experimental study of interrogating the trapping conditions of proteins by using DC-DEP is limited. To develop and demonstrate the capabilities of isolating proteins by DC-DEP, eGFP is introduced into the microfluidic devices that can generate increasing DEP forces with applied voltages. Our preliminary data have shown that the concentrated streamline is formed in the channels at the specific applied voltage and buffer condition by using DC-DEP. Through the calculation of concentration distributions, the DEP mobility can be estimated by the software COMSOL, and the corresponding DEP force can be further calculated as a result. The result of DEP force obtained based on the software will be compared/contrasted to the results derived from the evolving theories.

(Tu-P20) A Combined Near-Infrared and Mid-Infrared Spectroscopic Approach for the Detection and Quantification of Glycine in Human Serum

Thulya Chakkumpulakkal Puthan Veetil¹, Bayden Wood¹; ¹*Monash University*

Serum is fundamental for blood and nutrient transport and is an important matrix to monitor the health status of an individual as it contains a number of direct and indirect indicators of disease progression. However, several important diagnostic markers found in the circulatory proteome and the low-molecular-weight (LMW) peptidome have become analytically challenging due to the high dynamic concentration range of the constituent protein/peptide species in serum. Herein, we propose a novel approach to improve the limit of detection (LoD) of LMW amino acids by combining mid-IR (MIR) and near-IR (NIR) spectroscopic data using glycine as a model LMW analyte. This is the first example of near-IR spectroscopy applied to elucidate the detection limit of LMW components in serum. Moreover, for the first time, multimodal IR spectroscopy combining NIR and ATR-FTIR has been applied to determine the detection limit of a low-molecular-weight compound in human serum. The multimodal approach incorporating ATR and NIR spectroscopic approaches improves the detection limit of glycine in serum. The LoD was found to be 0.26 mg/mL with ATR spectroscopy and 0.22 mg/mL with NIR spectroscopy. Supervised extended wavelength PLS-R resulted in an RMSEP value of 0.303 mg/mL and an R^2 value of 0.999 over a concentration range of 0-50 mg/mL of glycine spiked in whole serum. The LoD was improved to 0.17 mg/mL from 0.26 mg/mL with the combined approach. In the future, we will exploit the proposed combinatorial spectral approach with Raman spectroscopy to assess the LoD of LMW proteins and amino acids. In summary, this proof-of-concept study shows that the detection limit of diagnostically dominant LMWF compounds can be improved using a combined near-IR and mid-infrared spectroscopic approach.

(Tu-P21) High-Throughput Droplet Microfluidic System For Antimicrobial Susceptibility Testing Of Antibiotics Against Common Drug-Resistant Bacterial Strains

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Over 2.8 million people in the United States suffer from antibiotic-resistant infections with over 35,000 deaths recorded annually. The currently available conventional methods of detecting bacterial infections limit early diagnosis and treatment. In this work, we develop a time-efficient approach for performing antimicrobial susceptibility testing of *E. coli* bacteria in ~ 2 hours in droplet microfluidic devices. We demonstrate the in-chip incubation of single bacteria with reporter molecules in sub-nL sized water-in-oil droplets. Our proposed approach to performing AST will eliminate the need for bacterial amplification or off-chip incubation and provide timely AST information on bacteria. The methods which will be developed herein can be extended to other bacterial types.

(Tu-P22) A Reversed-Phase High Performance Liquid Chromatographic Method for the Determination of Ceftriaxone in Human Plasma

Peter Tang¹; ¹*Cincinnati Children's Hospital Medical Center*

High-performance liquid chromatographic (HPLC) methods for the determination of ceftriaxone concentrations in biological fluids have been previously described which offer rapid, more specific, more accurate, and more reproducible results for both pharmacokinetic studies and routine clinical testing. Because ceftriaxone is a highly polar compound, adding an ion-pairing agent to the mobile phase would necessitate the retention of ceftriaxone on the analytical column. To avoid peak trailing which ceftriaxone exhibits in reversed-phase HPLC, the pH of the mobile phase would also be adjusted to 9, and a precolumn has to be used to prevent damage to the analytical column from this alkaline mobile phase. Although the addition of ion-pairing agent can improve the peak shape of ceftriaxone, disadvantages include the large volumes of mobile phase are required to equilibrate the analytical column, and damages to the analytical column by the quaternary ammonium salts of ion-pairing agents. Also, the high pH of mobile phase diminishes the life of analytical column. In this report, a true reversed-phase HPLC with ultraviolet detection (UV) is developed and validated for the determination of ceftriaxone in human plasma. In contrast to earlier HPLC methods, the current method requires no addition of ion-pairing agent to the mobile phase. Sample preparation was performed by simple protein precipitation of 100 μ L plasma sample. The chromatographic separation of ceftriaxone and internal standard 8-chlorotheophylline was performed on an Agilent Zorbax Eclipse Plus C18 column with 50mM potassium dihydrogen phosphate buffer (pH 4.6) and acetonitrile as mobile phase at a flow rate of 1 mL/min with a total run time of 10 minutes. Both the analytes were monitored at a wavelength of 270 nm. The developed HPLC-UV method had the acceptable symmetrical peaks, good resolution, and analytes were eluted with good retention times. Linearity, precision, and accuracy were found to be acceptable over the concentration range of 1-200 μ g/mL for ceftriaxone. The developed HPLC-UV method can be used for the measurement of ceftriaxone concentrations in human plasma.

(Tu-P23) Can Radiolabeling Techniques Reveal Interferon- β 's Mechanism of Action in Patients with Multiple Sclerosis?

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In multiple sclerosis (MS), an autoimmune disease that affects the central nervous system, the myelin sheath surrounding axons is damaged, resulting in lesion formation and altered nerve signals. As with many autoimmune diseases, the mechanism of destruction is unknown; however, one proposed mechanism for demyelination involves excess nitric oxide (NO) production. Previous studies on MS red blood cells (RBCs) has shown an increase in RBC-derived adenosine triphosphate (ATP) release compared to healthy RBCs, which results in an increase in endothelial cell NO production. Additionally, the binding of an albumin/C-peptide/ Zn^{2+} complex has demonstrated an increase in RBC-derived ATP release, and we have shown that more albumin binds to control RBCs in the presence of C-peptide. Interferon- β (IFN- β) is an immunomodulating therapy used to treat MS, and while its mechanism of action is unknown, IFN- β has been shown to specifically bind to Zn^{2+} . We hypothesized that MS RBCs would also bind more albumin because of an increase of albumin/C-peptide complex receptors. This study analyzed the binding of radiolabeled bovine serum albumin (BSA- ^{99m}Tc) to control and MS RBCs in the presence of C-peptide and Zn^{2+} as well as IFN- β . There was a significant increase in albumin binding to MS RBCs (17,866 \pm 452 BSA molecules/RBC) compared to control RBCs (15,615 \pm 378 BSA molecules/RBC) in the presence of C-peptide and Zn^{2+} . There was a significant decrease in albumin binding to MS and control RBCs in the presence of IFN- β , C-peptide, and Zn^{2+} (13,538 \pm 314 BSA molecules/RBC and 14,462 \pm 223 BSA molecules/RBC, respectively). Meaning that IFN- β decreased albumin binding to RBCs by approximately 4,300 molecules in MS patients and 1,200 molecules in controls, making the albumin binding levels with C-peptide, Zn^{2+} , and IFN- β statistically the same in control and MS RBCs. This data, along with other data in our group, may explain the increase in RBC-derived ATP release and subsequent NO production that is observed in patients with MS. Overall, these data may provide insight into the need to dose IFN- β prior to meals so that the naturally occurring C-peptide and Zn^{2+} can assist in IFN- β 's mechanism of action.

(Tu-P43) Investigating Bacteriophage-Host Interaction Using Raman Spectroscopy Combined with Stable Isotope Labeling

ASIFUR Rahman¹, Wei Wang¹, Peter J. Vikesland¹; ¹*Virginia Tech*

The metabolic activity of bacteria is fundamental to many environmental processes, such as biogeochemical cycling, disease marker propagation, biofilm development, etc. Bacteriophages exclusively infect bacteria and affect their metabolism, which has important implications for phage-based biocontrol in several fields, such as public health, food safety, therapeutics, sustainable energy production, etc. Raman spectroscopy has become an essential tool for biomolecular analysis due to its sensitive detection and non-destructive application. Stable isotope labeling involves supplementing microorganisms with non-radioactive isotopes (e.g., ^{13}C , ^{15}N , ^{18}O) for incorporation of these isotopes into metabolically active microorganisms. The combination of Raman spectroscopy with stable isotope labeling has become a widely used technique to investigate biochemical processes. This study is the first to describe the application of Raman-stable isotope technique to

investigate the dynamics of Bacteriophage-Host Interaction. In this study, we investigated how viral infection affects the uptake of deuterium (D) isotope by the bacterial strains *Escherichia coli* C3000 and *Pseudomonas syringae*. Both strains were observed to have a C-D stretching vibration peak (2100-2300 cm^{-1}) in the Raman spectrum when cultured in a D-labeled growth media. Bacterial growth, as well as the C-D peak intensity, were monitored following exposure to organism-specific bacteriophages (MS2 and Phi6, respectively). The average of the absolute Raman intensities of the C-D band of the viral infected bacteria samples was $\sim 3\times$ lower for MS2 infection and almost $\sim 22\times$ lower for Phi6 infection, when compared to the control with no virus infection. These results suggested that viral infection leads to a reduction of bacterial growth, hence resulting in inhibited metabolic activity and D uptake. The time-dependent Raman spectra of the MS2 and Phi6 infected bacteria *E. coli* C3000 and *P. syringae* showed the intensity of the C-D signal over time, which was consistent with their OD_{600} growth kinetics. A mostly linear correlation was observed between the absolute intensities of the C-D peak in the Raman spectrum and the initial \log_{10} concentrations (PFU/mL) of the bacteriophages Phi6 and MS2. Overall, the Raman-stable isotope technique can be a promising approach to examining the effects of environmental factors, such as viral infection on bacterial growth.

(Tu-P44) Analysis of Infection Steps of Virus with Culturing Cells by Raman Spectroscopy to Detect Viruses

Keita Iwasaki¹, Kazuto Takami¹, Momoko Imai¹, Kosuke Hashimoto¹, Hidetoshi Sato¹;
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Early detection of pathogenic viruses is important to prevent pandemics, as it leads to quick lockdown and also species identification for medicine design. At present, in order to detect pathogenic viruses, base sequence, antigen-antibody information and their amplification are necessary. In terms of amplification, it takes time not only for PCR detection but also for virus replication to proceed until the patient shows symptoms. The antigen or antibody detection method with immunolabeling also takes time for operation and reagent. As a non-conventional approach, we are studying a method using Raman spectroscopy to utilize living cells as a sensor for viruses.

Raman spectroscopy can non-invasively obtain molecular information without labeling, which reflect the intracellular molecular composition. We believe that Raman spectroscopy can be applied to real-time monitoring of pathogenic viruses using cultured cells. Previous studies reported that cultured cells infected with a viral vector that does not carry the target gene showed the intracellular molecular composition change, which reflected in the loadings of partial least square regression – discrimination analysis. Notable point is that the study showed potential to detect virus within a minimum of 3 hours after infection process. There are probably different cellular response depending on several manners of replicating nucleic acid information (DNA/RNA, single/double strand) and combining the presence/absence of envelopes and proteins related in adsorption with host cells. To apply this technique to real application, we should study various kind of viruses.

Therefore, we aim to detect intracellular responses common and specific cellular responses to virus species. Chemometrics and statistical processing is essential to extract

specific cellular response to time-dependent changes in adsorption, transcription, replication and particle formation after infection process along with the molecular compositional change depending cell cycle of host cells. Here, we report the results of comparing the intracellular responses when the same cell line is infected with a virus having a different infection-replication mechanism, such as lentiviral (single strand RNA with reverse transcriptase) and adenoviral (double strand DNA) vectors.

(Tu-P45) Simple Near-infrared Analysis of an Organic Phase Extracted from Bile Juice to Identify Gall Bladder Cancer

Yunjung Kim¹, Eunjin Jang¹, Hoeil Chung¹; ¹*Hanyang University*

(IR) spectroscopy was previously adopted for the identification of gall bladder (GB) cancer using bile as a specimen. An organic phase extracted from raw bile juice using a methanol;chloroform solution was dried on a Si wafer and then transmission IR spectrum of the dried sample was collected for the discriminant analysis. Based on the LC-MS analysis, the levels of lipids such as phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) in the GB cancer samples were lower. Therefore, the compositional difference was then reflected in the corresponding IR spectra and the IR-based identification of GB cancer was thereby feasible. By the way, a simpler and faster spectroscopic method such as without a sample-drying procedure is desirable for practical clinical application. For this purpose, near-infrared (NIR) spectroscopy has been readily explored for the direct analysis of bile-extracted chloroform phase. Since the molecular structure of chloroform is simple, chloroform absorption-free spectral ranges enabling of featuring out extracted lipids would be possible. With this expectation, NIR spectra of organic phases extracted from bile samples (obtained from normal, GB polyp, hepatocellular cancer and GB cancer subjects) were acquired and corresponding spectral features of the samples were comparatively analyzed. Finally, PCA was performed using all the samples spectra and the discriminability of GB cancer in the principal component (PC) score domain was assessed. In addition, NIR spectra of different lipids such as cholesterol, PCs, PEs, and fatty acid (FA) were also acquired by dissolving them in chloroform and compared with those of the employed samples.

(Tu-P46) Microfluidic Devices for Tracking Z-ring Dynamics in Response to Deletion of Negative Regulators in *Bacillus subtilis*

Laura C. Lastra¹, Yuanchen Yu¹, Daniel Kearns¹, Stephen C. Jacobson¹; ¹*Indiana University*

FtsZ is a tubulin-like protein that polymerizes into a ring structure (the Z-ring) at the site of future cell division. This ring provides the framework for interactions between many divisome proteins and for assembly of the division machinery. The central role of the Z-ring in cell division can be strategically used as a visual indicator of the progression of cell division when FtsZ is fluorescently labeled. How the ring is invariably positioned at the mid-cell where septum formation and cell constriction occur has been a matter of constant research.

Beyond nucleoid occlusion, which ensures the ring assembles between nucleoids, other divisome proteins are known to have a role in the spatiotemporal regulation of the ring. Particularly, the proteins EzrA and MinD have been identified as regulators of the ring by preventing assembly of extra Z-ring at the cell poles, where nucleoid occlusion is weak. One question is why these two proteins seem to have an overlapping function, yet do not share any homology and their deletion phenotypes diverge in that only a MinD deletion results in productive cell division and, consequently, minicell formation. Here, we track FtsZ parameters and localization of the Z-ring in *Bacillus subtilis*, at both the single cell and population levels in response to deletions of divisome proteins, EzrA and MinD. We make use of a hybrid glass-poly(dimethylsiloxane) device with an integrated microchannel array that confines bacterial growth to a single dimension, while allowing for extensive control over the environmental conditions. Our microfluidic device serves as a reliable platform to study interactions between the divisome proteins and how their interaction regulates Z-ring dynamics throughout the cell cycle, as phenomena that are hidden under the complex cultivation conditions of agar-pad based microscopy methods are revealed.

Tuesday Poster Session - CHEM

(Tu-P24) Back to the Drawing Board: A Unifying First-Principle Model for Correlating Sample UV–Vis Absorption and Fluorescence Emission

Max C. Wamsley¹, Samadhi N. Nawalage¹, Juan Hu², Willard Collier³, Dongmao Zhang¹;
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The popular textbook and literature model $I(\lambda_x, \lambda_m) = \boxed{\times}$ or its variants for correlating the sample absorption and fluorescence often fails even for the simplest samples where the

fluorophore is the only light absorber. Reported is a first-principle model $I(\lambda_x, \lambda_m) = \boxed{\times}$ for correlating the sample fluorescence measured with a conventional spectrofluorometer and its UV–vis absorbance quantified with a conventional UV–vis spectrophotometer. This model can be simplified or expanded for a variety of fluorescence analyses. First, it enables curve-fitting fluorescence intensity as a function of the fluorophore or sample absorbance over a sample concentration range impossible with existing models. Second, it provides the theoretical foundation for an inner-filter-effect (IFE)-correction method developed earlier and explains mathematically the linearity between the IFE-corrected fluorescence and the fluorophore concentration or absorbance. Third, this model can be expanded for quantitative mechanistic studies of fluorescence intensity variations triggered by stimuli treatments. One demonstrated example is to quantify temperature effects on the emission-wavelength-specific and total fluorescence quantum yield of anthracene. We expect that this first-principle model will be broadly adopted for both student education that promotes evidence-based learning and a variety of fluorescence applications where disentangling sample absorption and emission are critical for reliable data analysis.

(Tu-P25) Integrating-Sphere-Assisted Resonance Synchronous Spectroscopy for Quantification of Materials Double-Beam UV-vis Absorption

Pathum D. Wathudura¹, Max C. Wamsley¹, Juan Hu², Dongmao Zhang¹; ¹*Mississippi State University*, ²*Depaul University*

Integrating spheres (IS) have been used extensively for characterization of light absorption in turbid samples. However, converting the IS-based sample absorption coefficient to the UV-vis absorbance quantified with double-beam UV-vis spectrophotometer is challenging. Presented herein is an integrating-sphere-assisted resonance synchronous (ISARS) spectroscopy method performed with conventional spectrofluorometers equipped with an integrating-sphere accessories. Mathematical models and experimental procedures for quantifying the sample, solvent, and instrument-baseline ISARS intensity spectra were provided. A three-parameter analytical model has been developed for correlating the ISARS-based UV-vis absorbance and the absorbance measured with double-beam instruments. This ISARS method enables quantitative separation of light absorption and scattering contribution to the sample UV-vis extinction spectrum measured with double-beam UV-vis spectrophotometer. Example applications of this ISARS technique is demonstrated with a series of representative samples differing significantly in their optical complexities, from approximately pure absorbers, pure scatterers, to simultaneous light absorbers, scatterers, and emitters under resonance excitation and detection conditions.

(Tu-P26) Photodynamic Behavior in Solid-State Vinyl Azides That Vary Due to the Flexibility of Substituents Upon Gas Release

Fiona J. Wasson¹, Nayera Abdelaziz¹, Anna Gudmundsdottir¹; ¹*University of Cincinnati*

The use of sodium azide for gas expansion in airbags is toxic and can cause chemical burns, hence exploring organic azide structures for the same application can provide healthier emergency airbags¹. Also utilizing photochemistry, solid-state organic molecules grown as crystals can perform spectacular dynamic behavior for use in metal-free soft robotics, switches, and actuators as a sustainable option in carbon-nitrogen bond formation². Exploring vinyl and aryl azides as crystals for irradiation with light will release nitrogen gas, causing crystals to crack, splinter, bend, jump, roll, and other movements³. These photodynamic movements can be correlated to the crystal lattice packing provided by X-ray single crystal structure determination. With the confinements of the solid packing, reactions progress with proximity to other atoms coupled with gas releasing at the weakest interactions. To compare the packing and movements, computation calculations are completed for the activation energy of the reaction species and intermolecular force fields. Two vinyl azides (3-azido-1-indenone and 3-azido-5-methoxy-1-indenone) had the same photo mechanism when irradiated, while their dynamics differed widely⁴. The methoxy substituted bend while the non-substituted crack like sliced bread, both with 400nm light and within 10 seconds. The force field calculation explains the difference in flexibility and brittleness in the similar species.

(Tu-P28) Adapting Models from a Source Calibration Set to a Target Deployment Domain with Repeat Spectra or a Constant Analyte Sample Target Set

Jordan Peper¹, John H. Kalivas¹; ¹*Idaho State University*

The primary goal of spectral multivariate calibration is to determine a regression vector that relates spectral responses to their respective analyte values. Numerous regression techniques exist to fit a model to a collection of source samples with labeled analyte values, such as partial least squares (PLS). Models formed with traditional calibrations can be used to predict unlabeled target samples, but a problem arises when target spectra and/or analyte amounts have shifted from the source domain in which the model was formed. Using transfer learning methods, source models can be updated to fit the target conditions and continue predicting accurately. A variety of model updating methods based on unlabeled target data exist but often possess several mathematical restrictions that limit their use. By leveraging target sample situations with constant analyte values, the presented model updating method, null-augmentation regression constant analyte (NARCA), bypasses constraints limiting other transfer learning methods. It was determined that NARCA works effectively for three target data sampling methods. These circumstances are repeat spectra from a homogeneous or heterogeneous sample, and constant analyte valued samples. Three NIR datasets were examined for these respective situations consisting of cow milk, cattle feed, and mango spectra. The cow milk and cattle feed datasets have spectral domain shifts in combination with shifts in analyte amounts between the source and target domains. The third dataset of mango samples maintains only spectral shifts relative to the source data. Results demonstrate that NARCA is an effective transfer learning method.

(Tu-P29) **Exploring Prenol as a Bioblendstocks Additive for Gasoline-type blendstocks**
Lorenzo Vega-Montoto¹; ¹*Idaho National Laboratory*

An increasing proportion of biofuels and/or bioblendstocks incorporation in commercial fuels could represent a transition towards the decarbonization of the transportation sector through the proposed electrification strategy. Any biofuel or bioblendstock cannot satisfy all ASTM required specifications. To meet the whole set of specifications, several components (blendstocks) are mixed together. In the case of bioblendstocks, a base fuel composed of oil-derived refinery blendstocks is required (e.g., naphthas from the distillation, fluidized catalytic cracking, isomerization, alkylation, reforming units, etc.). Results indicate an interplay among the properties of the bioblendstock and those of the base fuel that need to be consider for formulating such base fuel. Furthermore, the composition of the base fuel could be adjusted to maximize the value-added to bio-refiners, refiners, and blenders. This work also confirms the validity of the hypothesized composition of the base fuel, by blending in prenol as an example of bioblendstock and measuring octane, volatility properties, sulfur content, oxidation stability and existing gums. Prenol represents bioblendstocks characterized by low volatility, and a boosting effect on research octane number (RON) and on octane sensitivity (OS). The evaluated properties met measured ASTM D4814 specifications, with the exception of oxidation stability, which could be easily met by using one of the typical correcting additives. The advantages of using a base fuel composition and formulation adapted and adjusted to make the best of a given bioblendstock are discussed. Data driven modeling using well established chemometrics methods will unveil the chemical features and chemical families interacting with prenol to

improve motor and research octane numbers.

Tuesday Poster Session - LIBS

(Tu-P30) A Novel Platform For High-Speed, High-Resolution Laser Induced Breakdown Spectroscopy Imaging

Shayne M. Harrel¹, Jean-Michel Laurent¹, Antoine Varagnat¹, Adrian Tercier², Vincent Motto-Ros³; ¹*Andor Technology*, ²*Université Claude Bernard*, ³*Institut Lumière Matière*

We present examples of advanced LIBS Imaging enabled by the latest generation of fast gated cameras based on sCMOS technology, combined with the latest generation of ultra-stable kilohertz Q-switch diode lasers. These state-of-the-art detectors provide significant enhancement in terms of high acquisition rates and simultaneously high dynamic range compared to CCD, Interline or EMCCD-based gated detectors. It enables the development of advanced high throughput spectroscopy techniques, for example in the context of micro Laser-Induced Breakdown Spectroscopy (μ LIBS) imaging. Spatial resolution down to 10 μ m is achieved by incorporating an automated translation stage. Timing diagrams and triggering schemes for the translation stage and intensified camera are discussed. LIBS images with up to 4K definition (3840x2160 pixels), obtained in less than 3 hours, will be presented. Such results show the spatially resolved elemental distributions of Si, Fe, Cu, Al, Mg, Ca and Ag for several geological samples (Silicate, Pyrite, Chalcopyrite and Carbonate), demonstrating the high throughput potential of such an approach and the great reduction in experimental time while preserving chemical information integrity.

(Tu-P31) Remote Isotopic Analysis of Lithium in Solids by Femtosecond Filament-Laser Induced Breakdown Self-Reversal Isotopic Spectrometry

Kévin F. Touchet¹, Jose Chirinos¹, Zach Alvidrez¹, Changmin Kim¹, Xianglei Mao¹, Vassilia Zorba¹; ¹*Lawrence Berkeley National Laboratory*

Laser-induced breakdown spectroscopy (LIBS) is a promising technique for fast elemental analysis of solids at atmospheric pressure, without any laborious sample pre-treatment [1]. Due its all-optical nature, LIBS allows detection at standoff distances. However, transitioning to progressively longer distances is limited by diffraction, which prohibits enough energy to be delivered remotely to form a laser-induced plasma. A solution to the long-range propagation issue comes in the form of femtosecond filaments which can propagate over long distances with suppressed diffraction, as a result of the dynamic balance between self-focusing and ionization [1].

Recently, in situ isotopic analysis in extreme environments has attracted interest for applications in nuclear security, non-proliferation, and nuclear fusion, as well as in environmental science. However, LIBS isotopic analysis is generally limited by small shifts as compared to the significant spectral broadening of plasma emission lines at atmospheric pressure [1]. Laser-Induced Breakdown self-Reversal Isotopic Spectrometry (LIBRIS) was proposed to overcome this limitation for lithium isotopic analysis [2].

LIBRIS uses the linear dependence between the central wavelength of the unresolved isotopic peaks of lithium with the isotopic abundance of ⁶Li, which has been observed on emission

lines such as on the absorption dip. Isotopic analysis is possible thanks to the precise determination of the wavelength [2] of the line, which shifts with isotopic abundance. In this work, we used the combination of the femtosecond filamentation and LIBRIS for **remote isotopic analysis of lithium**. The filament was launched at 18 m and the spectroscopic measurements were taken at 20 cm and at 18 m away from the sample. We obtained absolute uncertainties on the ${}^6\text{Li}$ abundance of ± 2.1 at. % and of ± 4.3 at. % in close and far detection respectively.

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(Tu-P32) Isotopic Analysis of Glassy Uranium Samples by UV-LIBS

Kévin F. Touchet¹, Jhanis J. Gonzalez¹, Richard Russo¹, Vassilia Zorba¹; ¹Lawrence Berkeley National Laboratory

In situ isotopic analysis in extreme environments has attracted growing interest in nuclear applications, such as safeguards, non-proliferation efforts, and nuclear fusion. Laser-induced breakdown spectroscopy (LIBS) is a preferred technique for fast elemental analysis of solids at atmospheric pressure without any laborious sample pre-treatment. The optical spectrum of uranium is rich in atomic and ionic lines leading to low emission intensity and potential spectral interferences. Moreover, LIBS isotopic analysis is generally limited by the emission line's small isotopic shift compared to spectral broadenings induced by the Stark effect. To overcome this limitation, LIBS isotopic analysis of uranium could be performed under vacuum as a way to lower electron density thus lowering spectral width. Other approaches used to reduce the impact of line broadening are based on specialized data processing by means of chemometrics, or experimentally by selecting a longer delay, when the electron density of the plasma has decreased. Most of the uranium analysis by LIBS has been done using IR ns-lasers, while the U II line at 424.43nm with an IS of -24.8pm has been demonstrated to have a significant emission intensity and lead to good analytical performances.

In this work, we demonstrate the advantages of using a UV ns-laser to perform the isotopic analysis of glassy uranium samples at atmospheric pressure. The isotopic analysis was focused on the analysis of three uranium lines: atomic lines at 421.387 nm and 682.69 nm and an ionic line at 424.43nm. Each of these lines was selected based on their isotopic split, relative intensity, and proneness to interference. Under the conditions of this study, we observed improvement in the analytical results using the U I line at 682.69nm. The 682.69nm line has the strongest relative intensity and the fewest spectral interferences for the sample matrix tested. Through the combination of a UV ns-laser and this strong line, we obtained similar analytical performance to previous studies performed in our laboratory, with a precision of ± 2.4 at. % on the ${}^{235}\text{U}$ abundance, using only 160 pulses accumulated per sample instead of 1000 pulses.

(Tu-P33) Mass and Morphology of Yttrium Plasma as Function of Ablation Energy

Shealyn Chestnut¹, Mary Foster¹, Jonathan A. Merten¹; ¹*Arkansas State University*

Atomic absorption measurements of the laser-induced plasma remain uncommon. Less common still are whole-plasma maps of the atomic absorption, which may be a useful diagnostic given the heterogeneity of the LIP, particularly at early times. We have used atomic absorption spectroscopy to map the neutral and first ionized composition of the plasma with spatial and temporal resolution. Previous measurements have shown voids and gaps between the sample surface and absorbing regions at various times under different gases. Here, we track the morphology and mass of yttrium plasmas as a function of 1064-nm ablation laser energy under a near-atmospheric-pressure helium atmosphere.

(Tu-P34) Compact, Combined Laser-Induced Breakdown Spectroscopy (LIBS) and Raman System for the Detection and Investigation of Food Contamination. System Description and Preliminary Findings

Sungho Shin¹, Iyll-Joon Doh¹, Euiwon Bae¹, Bartek Rajwa¹, J. Paul Robinson¹; ¹*Purdue University*

According to the World Health Organization (WHO), food contamination is becoming a global concern. Specifically, the increasing abundance of microplastics and heavy metals is a major contributor to soil, water, and food contamination. Heavy metals have also been reported to accumulate on microplastic particles, resulting in unknown but potentially additional harmful effects. In general, food contamination is further classified as microbiological, chemical, and physical. Agricultural products can be chemically and physically contaminated at different points of the food supply chain, including farming/production, packing, shipping, storage, and distribution stages. Current food contamination detection methods include vibrational spectroscopies and mass spectrometry techniques, among others. Combined laser-induced breakdown spectroscopy (LIBS) and Raman spectroscopy system may offer a lower-cost alternative method for field-portable analysis of agricultural product contamination and adulteration. These two optical methods share similar benefits, including ease of use, simple sample preparation, real-time detection, the availability of a standoff detection mode, and portability. In a combined LIBS/Raman device, the elemental and molecular analysis can be executed concurrently or sequentially in situations such as detecting microplastics or determining heavy metal presence. In this study, the feasibility of a combined LIBS and Raman system for detecting food contamination was demonstrated. The prototype system contained a pulsed 10 mJ near-infrared laser, a 5 mW visible continuous wave laser, a shared optical train, and a spectrometer unit. In this study, three different polymers usually found in microplastic and two metal powders were used to spike food samples, including dairy products (cheeses) and spices. The Raman and LIBS analyses were conducted at identical spots on the tested sample surfaces. The results illustrate the wealth of combined spectral information and provide qualitative and quantitative data describing the presence of adulterants in the food

samples. The LIBS/Raman spectral fingerprints were analyzed using machine-learning methods. Our research demonstrates the utility of multimodal optical systems in agricultural and environmental research.

(Tu-P35) Laser-Induced Breakdown Spectroscopy as a Readout Method for Detection of Biomolecules Labeled with Photon-Upconversion Nanoparticles

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Although various readout methods are used to identify cancerous cells in immunohistochemistry (IHC) and immunocytochemistry (ICC), due to the limitations of widely used labels (e.g., time-consuming signal development, limited possibility of multiplexing or short-term stability) there is a need for alternative readout techniques. Laser-induced breakdown spectroscopy (LIBS) is an optical emission method providing fast (up to microseconds per single spectrum acquisition), multi-elemental and large-scale analysis. The so-called Tag-LIBS approach was already used as a readout method for determination of HER2 biomarker labeled with photon-upconversion nanoparticles (UCNPs). Through the detection of yttrium signal, which is principal constituent of UCNPs, clear differentiation between HER2-negative and HER2-positive cell pellets was shown. However further optimization is still necessary. In this work the laser ablation with the use of different laser pulse wavelengths (266, 532 and 1064 nm) and fluences were investigated. Furthermore, the single pulse (SP) and double pulse (DP) LIBS arrangements were compared. To focus wholly on analysis simplified sample processing was deployed and therefore UCNPs doped with erbium and ytterbium deposited on microscope slide were used as samples. The best lateral resolution trade-off and the best yttrium signal-to-noise ratio were obtained using the combination of collinear DP LIBS setting with two laser pulses operating at 1064 nm wavelengths. Obtained results confirmed that LIBS suggests a big potential to meet IHC and ICC readouts needs.

(Tu-P36) Using LIBS to Characterize MPEAs in Extreme Conditions

Nicholas E. Pugh¹; ¹*South Dakota School of Mines*

When operating in conditions that can cause the material to fail in unusual ways, it is important to know what is happening with the structure of the material. Multi-principal element alloys (MPEAs) and high entropy alloys (HEAs) are a class of material that are being used in these extreme environments. In this study, laser induced breakdown spectroscopy (LIBS) was used to analyze the spectrum of the MPEA AlNbTaTiZr. This data was analyzed to determine various properties of the MPEA. When measuring LIBS spectra data, various lighter elements can interact with each other to cause atoms to appear as though they have a higher energy state, although they do not. This is referred to as a “matrix effect”. It is important to determine when and where a matrix effect can occur with various laser-based

techniques, such as LIBS. The samples that were created and tested were either made from high-purity metals (Al, Nb, Ta, Ti, and Zr), or high purity equimolar concentrations of AlTi, AlNbTi, AlNbTiZr, and AlNbTaTiZr. LIBS spectra were taken for each sample. When observing two neutral peaks from the spectra, it was shown that there was a matrix effect when titanium was added to aluminum and vice-versa. Using the LIBS data, various materials properties can be extracted, such as the electron temperature and microhardness. The electron temperature was determined by the ratio of ratios method. A neutral and ionic peak were chosen for two different elements and, using these peaks, the electron temperature was determined. This study demonstrates that the microhardness of the alloy correlates with the electron temperature. Determining the microhardness from the electron temperature is useful as it allows for determining the microhardness of the material during operation of the extreme condition environments. Using LIBS during operation is vital to determine mechanical and chemical degradation in extreme environments. Knowing when and where molecules form will provide insight to any hazardous conditions, especially in fusion reactors. To accomplish this, molecular lines from the LIBS spectra were analyzed using PHOPHER simulation tool, where provides farther understanding of molecular species formation under extreme conditions.

(Tu-P37) Iron Measurement in Wastewater Outfall by Laser-Induced Breakdown Spectroscopy

CR BHATT¹, Daniel A. Hartzler¹, Dustin L. McIntyre¹; ¹*NETL*

In this study, laser-induced breakdown spectroscopy (LIBS), in conjunction with principal component analysis (PCA) and linear discriminant analysis (LDA), was used to determine iron content in coal ash runoff. Wastewater was collected over a period of 10 days from the foundation underdrain of a building built on top of a coal ash base and was analyzed using a traditional LIBS benchtop system and custom LIBS probe. Spectra obtained from the test samples were compared to iron containing standards to identify the prominent iron emission lines as characteristic signatures. The strong atomic emission lines of iron at Fe I 371.9 nm and Fe I 373 nm were identified with the help of these standard samples, which demonstrated the significant presence of iron in the collected wastewater samples. Machine learning tools, PCA and LDA were used to classify the liquid samples by collection day. For quantitative study, partial least square regression (PLS-R) calibration curves were developed using the data collected from the reference samples and were used to estimate the iron concentration in the wastewater samples. To evaluate the accuracy of the results obtained, they were compared with inductively coupled plasma-mass spectrometry (ICP-MS) measurements, and the results were found comparable with relative difference below 15%.

(Tu-P38) Increasing signal-noise ratio in laser-induced breakdown spectroscopy using a 3D-printed Ar(g)-flushed partial-vacuum chamber (ArVaC)

Sofia Paraoulaki de Miranda¹, Max Vallone², Victoria Paraoulaki de Miranda¹, Francisco J. Gomez Rivas-Vazquez¹, Claudia Ochatt¹, Robert C. DuBard¹; ¹*Ransom Everglades School*, ²*Ransom Everglades*

Laser-induced breakdown spectroscopy (LIBS) is usually conducted in open atmospheric conditions. Since particulates can interfere with ions, high background noise to signal ratios are obtained. This prototype provides an inexpensive argon-flushed, 3D-printed partial-vacuum housing solution compared to the 5-figure commercial vacuum chambers. Ar(g), an affordable noble gas, provides oxidation shielding during welding. Similarly, as LIBS samples are subjected to temperatures above 15,000 K for up to 10 ms, our design uses low-velocity Ar(g) streams to flush the testing surface of interfering particles while maintaining the integrity of the sample. An SLA photopolymer was used to 3D-print the housing, leaving one opening for the collimating lens, a second for the fiber optic outing, and a third for the gas valves. Gaskets seal all components to the housing, minimizing gas/vacuum loss during operation. A 0.25% low-reflectance, plano-convex quartz lens compatible with an Nd:YAG laser (1064/532 nm), capable of withstanding 10 J/cm², with a 100-mm focal length is incorporated into the housing with the convex side pointing to the laser beam, and the planar one to the sample. The second opening connects a T-manifold to the Ar(g) tank and a vacuum pump-connected Kitasato flask. This prototype is expected to reduce background noise and permit the detection of confirmatory secondary emission peaks, otherwise undetectable in open atmospheric conditions, for both sputtering solid standards and semi-fluid samples. This innovative system is functional in small spaces, inexpensive to construct, and increases the signal-to-noise ratio.

(Tu-P40) Developments in the Rapid Diagnosis of Bacterial Pathogens Using Laser-Induced Breakdown Spectroscopy

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Our lab has been investigating the use of laser-induced breakdown spectroscopy (LIBS) for the rapid detection and diagnosis of bacterial pathogens. LIBS is a spectrochemical technique that utilizes a laser to produce a near instantaneous elemental assay of a substance. The laser interacts with the substance to produce a high-temperature microplasma. As the plasma cools it emits light, which is collected by an Echelle spectrometer to give a high resolution time-resolved spectrum.

Currently we prepare bacteria samples by depositing them on a nitrocellulose filter through a custom fabricated centrifuge device and custom fabricated cone. The resulting thin film of bacteria is ablated in our LIBS apparatus. Using this sample preparation method, we have collected several hundred spectra of five species of bacteria: *Staphylococcus epidermidis*, *Escherichia coli*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*, to determine the ability to discriminate these organisms on the basis of their LIBS spectrum. We have also investigated the spiking of sterile clinical specimens of blood and urine with *E. coli*, *S. epidermidis*, and *E. cloacae* to determine the limits of detection, sensitivity, and specificity of a LIBS based clinical diagnostic test.

Detection and diagnosis of bacterial specimens based on a spectrochemical signal relies on the use of chemometric algorithms for spectral classification. Our work uses

discriminant function analysis (DFA), partial least-squares discriminant analysis (PLSDA), and we are investigating the use of artificial neural networks (ANN). In the past, we have achieved reliably high discrimination accuracy with pelletized bacterial targets containing high numbers of cells. However, detection and diagnosis of bacteria becomes increasingly more difficult as bacterial suspension concentrations decrease. In this poster we present ways to achieve reliable detection and diagnosis with lower and more clinically relevant concentrations of cells. The sensitivities and specificities achieved with methods focusing on optimizing discrimination will be reported for both the detection of cells in sterile fluids using PLSDA and the discrimination between bacterial species using DFA. Lastly, the use of an ANN algorithm will also be discussed.

(Tu-P41) A Customizable Modular Axes Positioning System (MAPS) for Laser-Induced Breakdown Spectroscopy

Victoria Paraoulaki de Miranda¹, Max Vallone², Sofia Paraoulaki de Miranda¹, Francisco J. Gomez Rivas-Vazquez¹, Claudia Ochatt¹, Robert C. DuBard¹; ¹*Ransom Everglades School*, ²*Ransom Everglades*

This modular axes positioning system (MAPS) was designed based on a five-axis positioning system (FAPS) (Vallone et al., 2019) to increase reproducibility and precision when analyzing sample emission spectra using laser-induced breakdown spectroscopy (LIBS). Remaining issues in FAPS include limited accuracy and repeatability due to coarse displacement as a result of the use of stepper motors and mechanical properties of the thermoplastic used in 3D-printed parts. MAPS addresses these issues while maintaining significantly low cost compared to commercial solutions. Improvements include: (1) exchanging the stepper motor-driven lead screw for a linear motor, (2) replacing 3D-printed thermoplastic structures with laser cut metal plates to increase tensility, and (3) direct encoder feedback. Axes that include linear motors, linear rails, and encoders are independently controlled and can be modularly stacked upon other axes. An additional value of the MAPS design is that the height (< 16 mm) does not compromise the vertical available space in the LIBS enclosure required for safe laser operation. The mounting plates, carriage assembly, and magnet plates were designed in *Fusion360* and laser-cut from 5052 aluminum. Spacers and mounts were manufactured using fused deposition modeling (FDM) and stereolithography 3D-printers. Experimental mounting of six axes using MAPS permitted control of planar distances (XYZ) of the sample on the mounting screen, the rotary axis of the sample relative to the laser (A-axis), the distance from the mounting screen to the fiber-optic (B-axis), and the distance between the plano-convex collimating lens and the mounting screen (C-axis). Desired coordinates are entered on a programmed Raspberry Pi (Shaked et al. 2021), which sends commands to the axis drivers. Preliminary data using this six-axis MAPS configuration results in minimum possible increments of 20 nm given by the linear encoder compared to the 12.7 μm stepper motor increments, a 3-orders of magnitude improvement in precision. This six-axis MAPS configuration can be in-house produced in under 80 hours of construction at less than \$2,000, $\frac{1}{5}$ of the cost of available commercial products, and provides superior performance in terms of precision,

durability, and repeatability.

**(Tu-P42) A 3rd Tuned PLS Model for Coal Property Analysis Using fs-LIBS System:
A Comparative Study to Industrial Coal Analyzer**

Sahar Sheta¹; ¹*Tsinghua University*

Spectroscopic data treatment with univariate models often leads to poor analytical results especially with materials with high matrix effects like coals. Partial least square (PLS) regression has been widely applied to maximize the linear correlation between the predicted and standard concentrations of the quantity of interest via producing a vector of weights that scalarly multiplied with the spectral components. Yet, however proved robust for several applications, PLS is criticized for the possibility of overfitting the data due to lack of physical-chemical interpretation of spectral information. Variables selection overcomes the downside in the statistical algorithm and improves the data understanding and model estimation/prediction interpretation. In this letter, a third-tuned PLS model is used to evaluate the quantitative performance of a LIBS system employing ultrashort laser pulses (fs-LIBS) for coal property analysis relative to industrially-applied nanosecond coal analyzer operates with optimized in-field parameters. The model was built to select/make use of correlating, informative, and stable lines (i.e. variables) within the spectra obtained by each system. First, Pearson's correlation coefficients are calculated showing lines most-correlated to each coal property, which among them, lines with physical-chemical relation are selected. This step proved useful for both understanding contrastive nature of fs and ns laser ablation mechanisms which yield different atomic/ionic lines or molecular fragments for correlation and in picking exceptions from abundant mineral lines correlating to ash content property. Second, the PLS is trained with full spectral information repeatedly with several randomly selected samples each time. The 100 lines with lowest RSDs of regression coefficients are chosen. This step incorporates stable, robust, statistical-based lines while eliminates those based on statistical artifact. Third, the two-step outcomes are taken as the input variables for PLS model to obtain results. Results show higher quantitative performance for fs-system over its ns-counterpart with RMSEPs decrease from 2.05% to 1.98%, 1.23% to 0.93%, and 2.70% to 1.18% for carbon content, heat value, and ash content, respectively. RMSECs show decrease from 3.34% to 2.45%, 1.22% to 0.97%, and 3.33% to 1.89% for same properties in order.

Wednesday, October 5, 2022

Oral Presentations

22AES03: Microfluid Electrokinetic Devices

Chair: Rucha Natu

Co-Chair: Josie Duncan

(AES-03.1) Screening Membrane Proteins in Microfluidic-Made Giant Unilamellar Vesicles

Adam Abate¹; ¹*UCSF*

Membrane transport proteins mediate the exchange of ions, small molecules, and macromolecules across the cell membrane. These important cellular gateways are involved in signaling, neurotransmission, metabolism and nutrient uptake across the tree of life. Despite their fundamental biological importance, the study of membrane proteins using directed evolution and deep mutational scanning remains challenging due to their requirement for the lipid membrane environment. We have developed a cell-free platform for high-throughput screening of membrane transport proteins. Our approach uses microfluidics to generate monodisperse giant unilamellar vesicles containing transporter genes and cell-free protein expression reagents. We apply our screening platform to analyze all mutants in the *de novo* designed transporter Rocker and identify a unique variant with enhanced kinetic properties. Our approach provides a general framework for high throughput experimentation of membrane proteins, including deep mutational scanning and directed evolution.

(AES-03.2) Electrokinetic Separation of Highly Similar Microparticles

Alaleh Vaghef Koodehi¹, Curran Dillis¹, Blanca H. Lapizco-Encinas¹; ¹*Rochester Institute of Technology*

We present a strategy for separating almost identical microparticles that can be extended to cells

Insulator-based electrokinetic (iEK) systems can provide an effective alternative for the analysis of micron-sized particles (including microorganisms) that could be analogous to long-established chromatography techniques for the analysis of nano-sized particles. There is a current need for effective separation techniques for microparticles. By taking advantage of the flexibility offered by iEK devices for combining linear and nonlinear EK phenomena within the same device, it is possible to design challenging separation of highly similar microparticles. In this work, by performing careful characterization of the EK behavior of the microparticles and building an accurate computational model using COMSOL Multiphysics® it was possible to predict the conditions for a successful separation. We achieved the separation of two almost identical microparticles by exploiting a tiny charge difference. The two types of particles separated here had the same size, and shape and were made from the same substrate material, and possessed the same type of surface functionalization. The selected polystyrene microparticles had a diameter of 5.1 μm and individual particle zeta potentials of 27.2 and 30.8 mV, thus a difference of only 3.6 mV. The experimental results were in good agreement with COMSOL estimations of retention time values; and had good reproducibility with variations of only 9% and 11% between repetitions. This charge-based binary separation illustrates that careful microparticle characterization and modeling are essential in designing challenging separation processes. The effective combination of linear and nonlinear EK effects can enable the discrimination and separation of almost identical microparticles.

Acknowledgments:

This material is based upon work supported by the National Science Foundation under Awards No. 1705895 and No. 2127592.

(AES-03.3) Combining Linear and Nonlinear Electrokinetic Effects in Microfluidic Devices

Blanca H. Lapizco-Encinas¹; ¹*Rochester Institute of Technology*

Microscale electrokinetic (EK) is a major pillar in the field of microfluidics, in particular for applications such as sorting and separation of particles, including macromolecules and cells. Many successful EK-based systems have been developed for the purification, enrichment, and isolation of a wide array of particles. EK keeps gaining popularity in the field of microfluidics because it is a label-free method that relies solely upon physical mechanisms, i.e., no chemical reactions are needed. Furthermore, it is possible to combine linear and nonlinear EK effects to achieve challenging separations.

Insulator-based EK (iEK) microdevices are systems that feature insulating structures that distort the electric field distribution within the device creating zones of higher field intensity. These zones of higher field intensity are where nonlinear EK effects arise and can be then used to finetune a desired particle separation. This present work is focused on the combination of linear and nonlinear EK effects for carrying out particle separations. We employ microchannels made from PDMS, that contain an array of insulating structures that alter the electric field distribution within the channel when an electrical potential is applied. We studied these systems with both, extensive mathematical modeling with COMSOL Multiphysics and careful experimentation. This presentation includes a summary of the latest developments from our laboratory, including the definition of the newly identified parameter of electrokinetic equilibrium condition (E_{EEC}) and a discussion of the importance of the nonlinear mechanism of electrophoresis of the second kind. Finally, we will highlight the distinct strategies employed to achieve efficient particle sorting and separation in just a few minutes.

Acknowledgments:

This material is based upon work supported by the National Science Foundation under Awards No. 1705895 and No. 2127592.

(AES-03.4) 3D-Printed Electrically Triggered Droplet Microfluidics for Reduced Sample Consumption During SFX

Diandra Doppler¹, Mukul Sonker¹, Ana Egatz-Gomez¹, Garrett Nelson¹, Mohammad Towshif Rabbani¹, Abhik Manna¹, Cole Errico¹, Jorvani Cruz Villarreal¹, Jose Manuel Martin Garcia², Rebecca Jernigan¹, Sahba Zaare¹, Konstantinos Karpos¹, Roberto Alvarez¹, Sabine Botha¹, Gihan Ketwala¹, Thomas Grant³, Angel Pey⁴, Alice Grieco⁵, Miguel Angel Ruiz-Fresneda⁵, Alexandra Tolstikova⁶, Reza Nazari¹, Uwe Weierstall¹, Valerio Mariani⁷, Petra Fromme¹, Richard Kirian¹, Alexandra Ros¹; ¹Arizona State University, ²Spanish National Research Council, ³University of Buffalo, ⁴University of Granada, ⁵Consejo Superior de Investigaciones Científicas, ⁶Deutsches Elektronen-Synchrotron, ⁷SLAC National Accelerator Laboratory

Revolutionary sample injection technique minimizing protein crystal sample waste in serial crystallography with XFELs.

X-ray crystallography is one of the most powerful tools for protein structure determination. With advances in X-ray free electron lasers (XFELs), serial femtosecond crystallography (SFX) enabled structure determination of challenging proteins like membrane protein complexes. Unlike traditional X-ray crystallography, in SFX the structure of a protein is determined by merging thousands of diffraction patterns from single nanometer-to-micrometer sized crystals that are irradiated by the intense XFEL pulse. Because of the serial delivery in SFX experiments, up to 99% of sample delivered to the X-ray beam is wasted due to the intrinsic pulsed nature of all XFELs. To solve this major sample consumption problem, we report a revolutionary sample-saving method which is compatible with all current XFELs.

To reduce the sample consumption during SFX, we had previously developed a device capable of generating and stimulating aqueous protein crystal droplets segmented by an immiscible oil before injecting the sample into the x-ray beam via a 3D-printed gas dynamic virtual nozzle (GDVN). Most recently, we designed a fully 3D-printed droplet delivery device where the phase and frequency of the droplets is optically detected and then shifted, through continuous electronic stimulation, to match the phase of the XFEL pulses.

These devices along with intricate data processing was implemented at the Macromolecular Femtosecond Crystallography (MFX) instrument at the Linear Coherent Light Source (LCLS) to deliver NAD(P)H: quinone oxidoreductase 1 (NQO1) and phycocyanin crystals. The data processing recorded droplets and electrically stimulated them to synchronize with the 120Hz pulses of the LCLS XFEL while an interface to the MFX EPICS data stream allowed direct comparisons of the droplet phase with the hit rate in the online data analysis monitor. Additionally, Python scripts were used to automate scanning of electrical feedback conditions. Optimized parameters resulted in a 7x increase in crystal hit rate. Furthermore, droplet injection reduced flow rates for sample to 4 $\mu\text{L}/\text{min}$, amounting in 75% sample conservation compared to continuous injection. Diffraction data obtained from this experiment resulted in the first room temperature structure of NQO1 to 2.5Å resolution showing distinctive structural features in the catalytic site not previously observed.

(AES-03.5) **Ionic Liquid Packed Microfluidic Device for the Selective Detection of CO₂**
Sreerag Kaaliveetil¹, Yun-Yang Lee², Ruth Dikki², Zhenglong Li¹, Yu Husan Cheng¹,
Charmi Chande¹, Burcu Gurkan², Sagnik Basuray¹; ¹*New Jersey Institute of Technology*,
²*Case Western Reserve University*

A hand-held gas sensor for sensitive and selective detection of ultra low gas concentrations

Development of miniaturized gas sensors with high selectivity and sensitivity still remains a challenge. One of the ways to achieve high sensitivity is by integrating microfluidic channels to the sensors, as it allows to analyze small volume of gases. High selectivity is then usually achieved by coating these microfluidic channels with different polymers. But this might compromise the sensitivity of the device. In this work we use an ionic liquids (IL), that is tuned to selectively capture CO₂, to increase the selectivity of our microfluidic device for CO₂ sensing. Our device consists of microfluidic channel sandwiched between top and bottom glass slides containing microelectrodes. The top and bottom glass slides are placed in such a way that the microelectrodes are present above and below the microfluidic channel i.e., a non-planar microelectrode architecture. The microchannel is filled with the IL exposed to different concentrations of CO₂. Here we used 1-ethyl-3-methylimidazolium 2-cyanopyrrolide ([EMIM][2-CNpyr]) as the IL, as it is shown to be a good sorbent for CO₂ at low partial pressures due to its chemical affinity. Electrochemical impedance spectroscopy (EIS) experiments revealed changes in the impedance signature caused by the absorption of CO₂. Our device architecture is adaptable for detecting other gases (biomarkers and toxic gases) by changing the IL tuned towards the target analytes.

22BIM04: Machine and Deep Learning for Biomedical Diagnostics

Chair: Thomas Bocklitz

Co-Chair: Oleg Ryabchykov

(BIM-04.1) Optical Microscopy for Enhancement and Automation of Antimicrobial Resistance Detection via Raman Spectroscopy

Oleg Ryabchykov¹, Kateřina Aubrechtová Dragounová², Ute Neugebauer¹, Jürgen Popp²,
Thomas W. Bocklitz²; ¹*Leibniz Institute of Photonic Technology*, ²*Leibniz Institute of Photonics Technology*

The frequency of bacterial infections that are resistant to available antibiotics rapidly increased in recent decades. The wide use of broad-spectrum and reserve antibiotics only further affects the effectiveness of antibiotic treatments in the future. Suppressing the spread of multi-resistant bacterial infections can be done by using targeted therapy instead of

broad-range antibiotics. Unfortunately, it takes up to three days to evaluate antibiotic susceptibility with routine diagnostic methods, which makes the immediate targeted therapy impossible.

We research the detection of germs and their antibiotic susceptibility via a combination of photonic techniques and machine learning[1]. The main advantage of Raman spectroscopy is that it enables the investigation of small sample volumes, prospectively down to single bacteria. Implementation of standardized data processing pipelines and the data-driven modeling enables detection of the pathogens, and subsequently, detection of the response to the treatment with different antibiotic concentrations. The absence of time-consuming steps, such as cultivation, can shorten the sample analysis time from days to hours[2].

We implement an automated multi-step analysis pipeline that uses optical microscopy and deep learning for the sample quality check and the bacteria presence, as well as the initial bacteria type classification. Based on the outcome of such initial analysis, further steps of the Raman spectroscopic investigation are performed. Such a multi-level detection approach is necessary due to the difference in response of different bacteria to antibiotics.

The analysis results include both the pathogen information and the results of antibiotic sensitivity testing, which provides sufficient information for administering the most suitable antimicrobial treatment to the patients with pinpoint accuracy.

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(BIM-04.2) Deep Learning in Digital Pathology Powers Biomarker Discovery and Optical Biopsy

Stephen T. Wong¹, Stephen T. Wong¹, Raksha Raghunathan; ¹*Houston Methodist*

Digital pathology coupled with machine learning and big data acquisition is paving the way to revolutionize pathology tissue examinations. The availability of whole slide imaging (WSI) and many other large area multiplex fluorescence microscopy imaging technology enables the digitization of patient specimens on glass slides to produce on-screen whole slide images quickly. The current paradigm is to digitalize *ex vivo* or biopsy tissue or

histopathologic images and apply computational algorithms, in particular machine learning and deep learning (ML/DL), to extract features or biomarkers for disease diagnosis. This presentation shows some use cases of ML/DL applications in powering digital pathology beyond diagnosis and into treatment and research. In addition, exogenous agents added to pathologic specimens may sometimes create unwarranted artifacts or cause phototoxicity and photobleaching. Label-free imaging or optical biopsy would have the potential to alleviate such issues; and its integration with effective ML/DL and endoscopy techniques will lead to *in vivo* digital pathology. Furthermore, coupling pathologic image analysis with omics studies will expand the scope of digital pathology for understanding disease mechanism.

(BIM-04.3) Virtual Assays of Unlabeled Tissues via Fluorescence Microscopy and Deep Learning

Hongda Wang¹, Hongda Wang¹; ¹*Pictor Labs*

Histological staining of thin tissue sections is an indispensable tool for biomedical research and drug development. It is also widely practiced for the diagnosis of various types of disease. However, the traditional histological staining workflow involves high-cost and labor-intensive procedures, which also produce enormous environmental impact. Here we present a framework for virtual histological staining of unlabeled tissue sections using autofluorescence microscopy and deep learning. The success of our framework was demonstrated by digitally replicating the H&E stain and special stains on various types of organs, including kidney, liver, lung, gland, etc., with consistent image quality. Beyond the visual comparison, blind studies led by board-certified pathologists also confirm the equivalent diagnostic value of the virtually staining images compared to their histochemically stained counterparts. Furthermore, we demonstrated the successful prediction of immunohistochemical (IHC) and immunofluorescence (IF) stains for characterizing human breast cancer and detecting brain neuronal degeneration. The virtual immunostaining allows for staining colocalization of structures and could lead to more frequent assessments. By eliminating the reagent and technician variations in chemical staining procedures, our technique generates consistent staining results across various tissue types, providing an ideal starting point for the downstream analysis algorithms. More importantly, the virtual staining technique inherently allows multiplex staining on the same tissue section due to its label-free and non-destructive nature, visualizes the exact same biological content under multiple different stains, while also reducing the volume of tissue samples needed to be collected from the patients. In summary, the presented virtual staining framework provides a label-free, cost-effective, high quality, and eco-conscious alternative to the traditional histological staining workflow, which will introduce a paradigm shift to the histological staining field.

(BIM-04.4) Integration of Raman spectroscopy and Automated Sampling for Real-Time Bioprocess Insights in Perfusion Cell Culture

Lee LEE Asplund¹, Stacy Shollenberger¹, Amy Wood¹, Allyson Caron¹, Rakesh Bobbala¹;
¹*MilliporeSigma*

Building chemometric model libraries as a starting point for inline Raman measurements

Mammalian cell cultures are widely used as the workhorse platform in the production of biological products including antibodies, other therapeutic proteins, and growth factors.

Particularly for bioprocessing, Raman spectroscopy has become attractive for PAT applications given its inherent properties such as non-contact, non-destructive, high molecular specificity, and weak water bands for good quality analysis in aqueous solutions. Given the increasing interest for robust process design, optimization, and control in emerging intensified and continuous upstream platforms, Raman spectroscopy provides a great potential for real-time and in-situ measurement of relevant cell culture critical process parameters (CPPs) and critical quality attributes (CQAs).

As a complimentary technique to Raman spectroscopy, new technology has emerged to enable automated, aseptic sampling. Automated sampling systems can take a sample from the bioreactor while maintaining its sterility and deliver the sample to a variety of analytical instruments for real-time analysis, without the need for human intervention.

In this talk we will present case studies on the implementation of Raman spectroscopy-based process monitoring in bench-scale CHO cell intensified seed trains (N-1) and dynamic perfusion (N) cultures. Additionally, we will discuss the value of combining automated sampling with Raman spectroscopy for increased sample frequency during chemometric model building, leading to more accurate inline and real-time measurement of CPPs and CQAs in the upstream bioprocess.

22CHEM03: Chemometrics Something Borrowed, Something New

Chair: Federico Marini

(CHEM-03.1) Variable Selection Tools for Multi-Block and Multi-Way Data

Federico Marini¹, Alessandra Biancolillo², Jean-Michel Roger³; ¹*University of Rome La Sapienza*, ²*University of L'Aquila*, ³*INRAE*

The possibility of selecting meaningful variables is a wide-debated topic and several variable selection (or feature reduction) approaches have been proposed into the literature, which are designed so to meet different purposes, e.g., reducing the number of total variables or understanding which variables contribute the most to the investigated system.

In this context, the Covariance Selection (CovSel) [1] approach provides a filter selection based on model parameters embedded in the model building. CovSel is conceived to select variables in regression and discrimination contexts, and it assesses the features' relevancy based on their covariance with the response(s).

In this communication, recently developed variable selection approaches for multi-block and multi-way data based on the CovSel paradigm will be presented.

Sequential and orthogonalized covariance selection (SO-CovSel) [2] is a multi-block technique similar to sequential and orthogonalized partial least-squares (SO-PLS), but the feature reduction provided by PLS is performed by CovSel and predictions are made by applying multiple linear regression on the subset of selected variables.

On the other hand, N-CovSel, proposes to extend the CovSel principle to N-way structures, by selecting features in place of variables. Three main questions are addressed to achieve this: (i) How to define a feature in a N-way array; (ii) How to define the covariance between a feature and a response Y; (iii) How to deflate a N-way array with regard to a selected feature.

The complete algorithm of N-CovSel will be presented and its theoretical properties discussed.

Both techniques will be exemplified by means of real and simulated data.

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(CHEM-03.2) Information Selection and Object Weighting as Potential Solutions to the Black Hole Effect in Bilinear Curve Resolution Based on Least Squares

Raffaele Vitale¹, Mohamad Ahmad¹, Marina Cocchi², Cyril Ruckebusch¹; ¹University of Lille, ²University of Modena and Reggio Emilia

Several application studies recently reported in literature have highlighted how least squares-based methods for Multivariate Curve Resolution (MCR), like Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS), might suffer from critical limitations when coping with datasets featuring so-called *minor* components, *e.g.*, chemical compounds observable in only a few (pure) pixels of a hyperspectral image. The root causes behind this particular issue (denominated *black hole effect*) have only lately been formalized: in fact, when addressing MCR problems by means of algorithms like MCR-ALS, if the number of analyzed data points is relatively large, the leverage of those that may be essential for a MCR-ALS factorization might become too low for guaranteeing its correctness. That being said, two possible options to overcome this limitation can be envisioned: one could i) select a reduced block of observations and – provided that such essential data points are preserved – perform a MCR-ALS decomposition on it in an attempt to retrieve meaningful spectral fingerprints for the pure components to be characterized, or, alternatively, ii) adjust specifically the weight of these relevant data objects so as to artificially increase their

influence within the MCR-ALS optimization procedure. In either case, this essential portion of the original dataset needs to be accurately identified.

In this presentation, an overview of the aforementioned black hole effect in bilinear curve resolution will be given. Information selection and object weighting will be introduced as potential solutions to circumvent such an effect and algorithmic details to perform these two operations in the framework of MCR-ALS will be provided. Their implications and potential benefits will be illustrated through simulated and real-world examples borrowed from the hyperspectral imaging domain where, commonly, even single experimental runs may lead to the generation of massive amounts of spectral recordings.

(CHEM-03.3) Data Fusion in Multimodal Spectroscopic Imaging: A Real Tool to Help Interpret Data

Ludovic Duponchel¹, Alessandro Nardecchia¹, Anna de Juan², Vincent Motto-Ros³, Michael Gaft⁴; ¹*University of Lille*, ²*Universitat de Barcelona*, ³*Institut Lumière Matière*, ⁴*Ariel University*

Laser-induced breakdown spectroscopy (LIBS) imaging is an innovative technique that associates the valuable atomic, ionic and molecular emission signals of the parent spectroscopy with spatial information. LIBS works using a powerful pulse laser as excitation source, to generate a plasma exhibiting emission lines of atoms, ions and molecules present in the ablated matter. The advantages of LIBS imaging are potential high sensitivity (in the order of ppm), easy sample preparation, fast acquisition rate (up to 1 kHz) and μm scale spatial resolution (weight of the ablated material in the order of ng). Despite these positive aspects, LIBS imaging easily provides datasets consisting of several million spectra, each containing several thousand spectral channels. Under these conditions, the current chemometric analyses of the raw data are still possible, but require too high computing resources. Therefore, the aim of this work is to propose a data compression strategy oriented to keep the most relevant spectral channel and pixel information to facilitate, fast and reliable signal unmixing for an exhaustive exploration of complex samples. This strategy will apply not only to the context of LIBS image analysis, but to the fusion of LIBS with other imaging technologies, a scenario where the data compression step becomes even more mandatory. The data fusion strategy will be applied to the analysis of a heterogeneous kyanite mineral sample containing several trace elements by LIBS imaging associated with plasma induced luminescence (PIL) imaging, these two signals being acquired simultaneously by the same microscope. The association of compression and spectral data fusion will allow extracting the compounds in the mineral sample associated with a fused LIBS/PIL fingerprint. This LIBS/PIL association will be essential to interpret the PIL spectral information, which is nowadays very complex due to the natural overlapped signals provided by this technique.

(CHEM-03.4) Applications of Classification Algorithms to Data from Portable Instrumentation

Caelin Celani¹, Karl Booksh¹, Jocelyn Alcantara-Garcia¹, Tyler Cople², James Jordan², William Johnston³, Amelia Speed⁴, Rachel McCormick¹, Olivia Jaeger⁵, Carolyn Chen⁶;

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Chemometrics applied to various portable instrumentation applications

The advent and growing popularity of handheld instrumentation is allowing for the expansion of the analytical laboratory to locations previously inaccessible. While a primary trade off of having field portability is diminished spectral resolution, the implementation of multivariate data analysis can be used to overcome some aspects of the decreased overall performance relative to benchtop instrumentation. The viability of portable instrumentation coupled with chemometric classification algorithms is shown across two distinct applications. First, successful classification of individual species of tropical hardwoods from the genus *Dalbergia* and its lookalikes are shown. In this analysis, elemental fingerprints of each hardwood are collected with laser induced breakdown spectroscopy (LIBS) under varying conditions – including low signal-to-noise (S/N) with many spectra and high S/N with fewer spectra – and distinguished with a series of classification algorithms, including Partial Least Squares Discriminant Analysis (PLS2-DA), k-Nearest Neighbors (k-NN), Classification & Regression Trees (CART), Random Forests (RF), and Support Vector Machines (SVM). It can be concluded from this study that not only is LIBS a viable handheld tool to identify wood species, but also fewer spectra of higher quality data modeled with SVM yields the best classification results. Second, historical textiles are analyzed with fiber optic reflectance spectroscopy (FORS) and x-ray fluorescence (XRF) and classified based on a flat classifier “decision rule.” The decision rule is created based on visible reflectance peaks and shows better clustering relative to other relevant wavelength ranges discussed in the art conservation literature. This cluster analysis problem shows that when dyeing textiles, chromophores are present in such small quantities that bulk properties such as textile color overwhelm any trace analysis signals. In addition, at least for textiles, XRF spectra presented in this work indicate that FORS inflection point does not correlate with elemental composition of the textile & mordant.

(CHEM-03.5) **Building Concordant Ontologies Using KNARM (KNowledge Acquisition and Representation Methodology)**

Hande Kucuk McGinty¹; ¹*Kansas State University*

A tutorial for a methodology that can be applied across disciplines.

Recently, the increase and convenience in computational power enabled a rapid demand and need for data science applications. Research and development projects are seeking ontologies and machine-operable standardized vocabulary for approaches regarding cheminformatics and bioinformatics research. A continuous effort exists for creating applications that use ontologies and knowledge graphs across fields. In my research and through my volunteering efforts at ontology development groups, I was able to generate methodologies for creating and evolving bio-medical, food, and agricultural ontologies as well as utilizing them for applications that use machine learning algorithms in the backend. In this talk, I will give an overview of current directions, challenges, and possible future

directions on building and evolving ontologies, and how using ontologies may help and accelerate the integration of knowledge representation across different domains.

22CTP/EARLY02: Strategies for Finding Balance

Chair: Karen Esmonde-White

(CTP-02.1) Balancing Life between Science, Entrepreneurship, Doing Good and Family

Rina K. Dukor¹; ¹*BioTools*

My childhood career dream was to be a Doctor, like my grandma, but an internship at Prof. Kiederling's lab at UIC changed all that. Doing research changes a person – it changed me. Every day I learned something new, every day there were more questions and every day I went to bed thinking about my projects. When I graduated with PhD and realized that the little-known technology / instrument that we built in the lab could change how chemists do their work, my entrepreneurship gene awoke and BioTools was born. I was young, had no business knowledge and really no business skills to create and grow a business. And I had no finances to bring a sophisticated new technology, VCD, to the market. But I had two things: passion for VCD and determination. So, I quit my amazing job and became an entrepreneur.

As a woman scientist with a brand-new business and active volunteerism, and as a new mom, I learned very fast that doing everything perfectly was very hard. How do I balance all my loves and passions?

I do not have a crystal ball or even a perfect advise but in this talk, I will share what I learned, the numerous mistakes I made, the things I think I did right, and how this 'balance' always needs recalibration because Life does not stay still.

(CTP-02.2) Surface Pressure: A Non-Perfect Guide to the Neverending Work-Life Balance

Luisa T. Profeta¹; ¹*Rigaku Analytical Devices*

Work-life balance. An elusive concept for scientists, especially given the demands of schooling, working towards career stability, and even the crazy concept of starting a family. In light of the COVID-19 pandemic, today's culture mentality of "don't stop till you drop" began generating problems for most people; struggling to work full time, while balancing the demands of schooling and other household responsibilities. Despite progress in mental health awareness (a key component in work-life balance), there still is an incredibly large chasm between the ideal work-life balance and what reality serves to the majority of scientists in the field, especially female colleagues. Unfortunately this discrepancy between the balance of work and life well-predates the extra stressors triggered by the pandemic.

This presentation hopes to foster ideas for the audience of how work-life balance is not simply a one-size-fits-all concept for most folks, and honor the nuances and variances that each scientist needs to consider as they work towards an improved work-life balance. Internal and external pressures experienced in working towards a healthier, but never perfected work-life balance, will be addressed; and solutions (or failures) elaborated on from a mid-career, parent of three kids viewpoint.

(CTP-02.3) Managing the Early Career Transitions in Academia

Ishan Barman¹; ¹*Johns Hopkins University*

Transitions are challenging. Academic transitions are no exceptions. This talk presents the distillations of my own experiences navigating what can be a complex and uneven landscape (of known unknowns and unknown unknowns), yet form the basis of a truly rewarding phase. The myriad shifts – some dramatic, others subtle – offers exciting opportunities while the journey getting there courses through strange new lands. I will attempt to mix some practical suggestions on finding a balance with thoughts on the emotional aspects to the transitions.

(CTP-02.4) Mid-Career Challenges for the Sandwich Generation

Karen A. Esmonde-White¹, Mary Lewis¹, Ian Lewis¹; ¹*Endress+Hauser*

Ah middle age! The age where smile lines develop, mandatory health tests become more invasive, and high-speed motorized vehicles become strangely appealing. Mid-career status can be characterized by age (typically 35-45), by time after earning a terminal degree (>10 years post-degree), or by career stability, responsibilities, and progression. A mid-career scientist defies neat categorization based on our experiences and observations. Non-traditional educational paths, return to the work force, developing new proficiencies to move into a completely different field, or hybrid work situations are now commonly observed. Mid-career is also a point where personal responsibilities and professional duties are demanding and can conflict. Being at a mid-career stage is rewarding and brings a sense of accomplishment, confidence, and new challenges. We will discuss our personal experiences in finding balance between personal, volunteer, and professional demands. Our non-traditional career paths, volunteer work, and attempts to find balance can perhaps serve as good examples or cautionary tales.

(CTP-02.5) Panel & Open Discussion

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22IR05: Quantum Cascade Lasers for Chemical Sensing

Chair: Bernhard Lendl

Co-Chair: Pietro Patimisco

(IR-05.1) Trace Water Detection in Organic Solvents by Photothermal Spectroscopy using a Mach-Zehnder Interferometer

Giovanna Ricchiuti¹, Alicja Dabrowska¹, Davide Pinto¹, Georg Ramer¹, Bernhard Lendl¹;
¹*TU Wien*

We present an indirect mid-IR sensing scheme based on a photothermal spectroscopy (PTS) setup for the analysis of liquids on the example of sensitive detection of water traces in organic solvents (ethanol and chloroform) and as a most difficult application in aircraft's jet fuel. Our sensor system works reagent-free and can be applied in an on-line format in the chemical industry as well as for fuel quality control, being industrial applications where traces of water need to be accurately determined, preferably in real time. It thus holds great promise to replace the off-line Karl-Fischer titration method which is the current standard method for this application, but which entails important drawbacks such as being time consuming, requiring toxic reagents and producing waste. [1]

The PTS signal is directly proportional to the laser emission intensity of a modulated mid-IR excitation source and to the sample absorption: absorption leads to a periodical heating and cooling which leads to expansion and a sample refractive index change (Δn). [2] We use a second laser source to probe the consequent refractive index change (Δn). The challenge lies in detecting the smallest Δn . Our approach consists in an interferometric configuration. In particular, our liquid PTS sensor exploits a Mach-Zehnder Interferometer (MZI) able to sense sub-nm phase shifts $\Delta\phi$ between its two arms. In detail, we use a HeNe probe laser and an external cavity (EC)-QCL pump laser tuneable (1730-1565 cm^{-1}). The stability and linearity of our system are ensured by temperature stabilization and holding the MZI in its quadrature point using a PID controlled piezo electric transducer glued directly on a mirror in one arm of the MZI. When benchmarking the system against commercial FTIR spectrometers it is shown to be in excellent agreement with regards to band shapes and positions. Achieved limits of detection are in the low ppm region.

References:

- [1] N. Dantan, 'Determination of water traces in various organic solvents using Karl Fischer method under FIA conditions', *Talanta*, vol. 52, no. 1, pp. 101–109, May 2000, doi: 10.1016/S0039-9140(00)00328-3.
- [2] S. E. Bialkowski, *Photothermal Spectroscopy Methods for Chemical Analysis*. John Wiley & Sons, 1996.

(IR-05.2) Solvent Absorption Compensated Quantum Cascade Laser Infrared Microscopy for Bioimaging

Yow-Ren Chang¹, Seong-min Kim¹, Young J. Lee¹; ¹*National Institute of Standards and Technology*

We developed an infrared microscope that overcomes water absorption and enables hyperspectral imaging of cells

Fourier Transform Infrared (FT-IR) microscopy is a powerful vibrational imaging tool with the potential for quantitative chemical analysis and label-free imaging of biological materials. Recently, the availability of Quantum Cascade Lasers (QCL) as mid-IR sources has led to the design of new discrete frequency IR microscopes and improvements in IR imaging quality and chemical sensitivity. However, both traditional FT-IR and QCL-IR instruments suffer from poor signal-to-noise when analyzing proteins in water due to the strong water absorption near the 1650 cm^{-1} Amide I absorption peak. Recently, we demonstrated a solvent absorption compensation (SAC) technique that improves the signal-to-noise of IR measurements of hydrated biomolecules by dynamically adjusting the intensity of light to compensate for water absorption. In this work, we integrated the SAC technique into a home-built, transmission-mode QCL-IR microscope. We demonstrate the capability to acquire hyperspectral IR absorption images of hydrated fibroblast cells in the $1580 - 1724\text{ cm}^{-1}$ range while maintaining high signal-to-noise. This work enables detailed chemical analysis of biomolecules in cells and could be utilized for quantitative concentration measurements at a single-cell level.

(IR-05.3) QCL Based Mid-IR Dispersion Spectroscopy of Liquids

Bernhard Lendl¹, Alicja Dabrowska¹, Andreas Schwaighofer¹; ¹TU Wien

Mid-IR dispersion spectroscopy is an attractive, novel approach to liquid phase analysis that extends the possibilities of traditional methods based on the detection of absorption via intensity attenuation. This technique detects inherent refractive index changes (phase shifts) induced by IR absorption. In contrast to classic absorption spectroscopy, it provides extended dynamic range, baseline-free detection, constant sensitivity, and inherent immunity to power fluctuation. In this talk, a detailed experimental and theoretical characterization and verification of this method with special focus on broadband liquid sample analysis will be provided. For this purpose, we use the latest generation, compact benchtop dispersion spectroscopy setup based on an EC-QCL coupled to a Mach-Zehnder interferometer. Phase-locked interferometric detection enables to fully harness the advantages of the technique. By instrument operation in the quadrature point combined with balanced detection, the full immunity towards laser source power fluctuations and the environmental noise can be achieved. On the example of ethanol (0.5-50% v/v) dissolved in water, it is experimentally demonstrated that changes of the refractive index function are linearly related to concentration for strongly absorbing, highly concentrated samples beyond the validity of the Beer-Lambert's law. Characterization of the sensitivity and noise behavior indicates that the optimum applicable pathlength for liquid analysis can be extended beyond the ones applicable for absorption spectroscopy. Experimental demonstration of the advantages over classical absorption spectroscopy illuminates the potential of dispersion spectroscopy as upcoming robust and sensitive analytical method.

(IR-05.4) **Diffraction-Limited Mid-Infrared Hyperspectral Ellipsometry**

Markus Brandstetter¹, Alexander Ebner¹, Markus Brunner¹, Robert Zimmerleiter¹, Kurt Hingerl²; ¹*Research Center for Non-Destructive Testing GmbH*, ²*Center for Surface and Nanoanalytics, Johannes Kepler University*

The recent introduction of quantum cascade lasers as mid-infrared (MIR) light source in spectroscopic ellipsometry led to a leap in performance [1]. The high spectral brightness of QCLs enabled a significant improvement of signal-to-noise ratio and measurement speed compared to state-of-the-art Fourier-transform infrared ellipsometry. In addition to the exceptional spectral brightness, the laser emission of QCLs facilitates spot sizes that enable the acquisition of hyperspectral ellipsometric images at a microscopic level.

In this contribution we demonstrate a high-resolution hyperspectral MIR ellipsometry setup with an acquisition time for a single spectrum of less than 1s and a spatial resolution at the diffraction limit. Thereby, both measurement speed and spatial resolution exceed the state-of-the-art by orders of magnitude. The high time resolution of the system is exploited to directly monitor dynamic stretching processes of polymer films, revealing the reorientation of molecular chains [2]. The achieved spot size and spatial resolution was determined by means of a knife edge method and the investigation of test targets, respectively. The imaging capabilities of the fast MIR hyperspectral ellipsometry system are experimentally demonstrated by measurements of different inhomogeneous and spatially structured specimens. Samples have been investigated in both ellipsometric point-and-shoot scenarios as well as mapping procedures leading to hyperspectral Δ, Ψ -data cubes with over 300 spectral channels in each of the acquired 64×64 (4096) spatial pixels.

[1] A. Ebner, R. Zimmerleiter, C. Cobet, K. Hingerl, M. Brandstetter, and J. Kilgus, *Optics Letters*, 44 (2019)

[2] A. Ebner, R. Zimmerleiter, K. Hingerl, M. Brandstetter, *Polymers*, 14 (2022)

(IR-05.5) **Spectroscopic Applications of Quantum Cascade Laser Arrays**

Chu C. Teng¹, Christian Pfluegl¹; ¹*Pendar Technologies, LLC*

Pendar uses quantum cascade laser arrays to build broadband multi-species gas sensors.

Distributed feedback quantum cascade lasers (DFB-QCLs) are well suited for laser absorption applications owing to their compactness, high brightness, and high spectral purity. However, individual DFB-QCLs are limited to a few wavenumbers of spectral tunability, which prevents them from performing detection of multiple analytes or broadband absorption features. In contrast, Pendar's proprietary monolithic DFB-QCL arrays allow instant access to any QCL in the array, and broadband tuning is realized through successively activating

QCLs emitting different wavelengths. We further utilize electrically-induced frequency down-chirp to complete the spectral gap between array elements and realize a truly continuous spectral coverage with a tuning speed of more than $4 \text{ cm}^{-1}/\mu\text{s}$ and a resolution better than 0.05 cm^{-1} .

This talk will provide an overview of the sensing applications that leverages the monolithic DFB-QCL array, including miniaturized point sensing, mobile platform sensing, and broadband biomedical imaging. In particular, we will focus on a recent system implementation that applies the laser array to open-path long range gas sensing. The prototype sensor demonstrated multi-species sensing in the spectral region of 930 cm^{-1} - 1500 cm^{-1} with a frequency resolution of $< 0.05 \text{ cm}^{-1}$. Outdoor remote sensing was performed using a retroreflector with over 250-meter optical pathlength. Ongoing work focuses on improving sensor stability, extending the sensing range to 1 km pathlength, and delivering a compact, battery-powered, and fieldable system.

22IR09: Spectroscopic Methods for Materials Characterization

Chair: Richard Bourne

Co-Chair: Mike George

(IR-09.1) Raman Spectroscopy of Individual Electrospun Fibers

Christian Pellerin¹, Arnaud W. Laramée¹, Clarence Allen¹; ¹*Université de Montréal*

Better understanding of structure-properties and first polarized resonance Raman on individual fibers

Electrospinning can prepare polymer nanofibers that find application in tissue engineering, selective filtration, optical materials, etc. Electrospun fibers often show an exponential increase in mechanical, electrical, and optical properties at small diameters that has been associated to molecular orientation. Our group uses polarized confocal Raman microscopy to probe the orientation and structure at the individual fiber level. As discussed in this presentation, our results indicate that molecular orientation does increase exponentially at small diameters for amorphous or weakly crystalline polymers.¹ However, this trend is lost for highly crystalline polymers, where Raman shows high orientation at all diameters.² This high orientation can be exploited to impart useful functional properties to semi-conducting polymers. In particular, we will show by resonance Raman that the structure and orientation of poly(3-hexyl thiophene) can be optimized when preparing fibers in an amorphous or highly crystalline matrix.

1. A.W. Laramée, C. Lanthier, C. Pellerin, *Raman Investigation of the Processing – Structure Relations in Individual Poly(ethylene terephthalate) Electrospun Fibers*, **Applied Spectroscopy** 2022, 76, 51-60

2. A. W. Laramée, C. Lanthier, C. Pellerin, *Electrospinning of Highly Crystalline Polymers for Strongly Oriented Fibers*, **ACS Applied Polymer Materials** 2020, 2, 5025-5032

(IR-09.2) **Imaging the 3D Orientation of Polymer Chains by 2D Polarization IR Microscopy**

Young J. Lee¹, Shuyu Xu²; ¹*National Institute of Standards and Technology*, ²*NIST*

The first imaging of 3D orientation of continuously distributed molecules using 2D polarization IR microscopy.

Despite the ubiquitous occurrence of anisotropic molecular alignment in 3D, conventional imaging approaches based on polarization can map only molecular orientation projected onto the 2D polarization plane. I present a new algorithm to convert 2D polarization IR spectral data into the 3D angles of molecular orientations. The polarization-analysis algorithm processes a pair of orthogonal IR transition-dipole modes concurrently. The orthogonal-pair polarization IR (OPPIR) method maps the 3D orientation angles and the order parameter of the local orientational distribution of polymer chains in a thin polycaprolactone (PCL) film. The OPPIR imaging results show that polymer chains in the semicrystalline film are aligned azimuthally perpendicular to the radial direction of a spherulite and axially tilted from the surface normal direction. The 3D orientations of PCL chains in a quiescent spherulite region are compared to those in a shear deformed region of the same PCL film. In contrast to the quiescent area, polymer chains in the sheared area are azimuthally aligned along the shear direction but axially tilted from the shear direction. The unexpected out-of-plane tilted orientation indicates that orientational relaxation followed shear deformation and occurred predominantly in the out-of-plane direction. This newly available information on the local alignments in continuously distributed molecules helps understand the molecular-level structure of highly anisotropic and spatially heterogeneous materials.

(IR-09.3) **Trimodal Microscopy for Better and Faster Microplastic Identification IR + Raman + Fluorescence**

James R. Anderson¹, Mustafa Kansiz¹, Eoghan Dillon¹; ¹*Photothermal Spectroscopy Corp*

Trimodal technique like O-PTIR + Raman + Fluorescence for more robust, repeatable analysis, of microplastics

Microplastic (MP) contamination grows and the problem is now being recognized outside of scientific circles reaching mainstream media. Articles and stories have been posted on the “The Weather Channel”, in the “New York Times”, “LA Times”, plus other news outlets. California is the first state/locality to initiate new regulatory standards for microplastics aimed at measuring MP contamination in drinking and surface waters. Although the concern and need for a robust, fast and easy method for MP is needed, there are limits to quality and sizes of MP’s that can be tested.

The lack of a robust method for MP characterization is often hampered by the tools most readily available to characterize and chemically specify MP’s. FTIR micro spectroscopy systems are often utilized but suffer from spatial resolution and scatter artifacts for MP’s. Some studies aimed for a size limit of 10-20 μm but in practice loosened the requirement because robust characterization is impeded below 50 μm .

A new approach to IR micro spectroscopy, called “Optical Photothermal Infrared (O-PTIR)” spectroscopy has demonstrated a unique ability to generate submicron IR spectra, in reflection mode without common scatter artifacts of direct IR measurements using FTIR or new QCL systems. The O-PTIR technology uses a pump (IR laser) -probe (vis/NIR laser) that provides the ability to measure mm’s to submicron MPs with infrared chemical specificity in a non-contact reflection geometry. The O-PTIR spectra are comparable to FTIR transmission spectra making them easily searched in common IR databases. Since the vis/NIR laser used is a high quality Raman grade laser it can provide for the simultaneous acquisition of IR and Raman spectroscopy from the same spot with the same submicron resolution.

The outlook for not only improved MP identification accuracy is possible with mIRage but we are also looking to improve MP throughput. Recently we have developed the coupling of fluorescence microscopy to deliver “Fluorescence-guided O-PTIR”. This uses the well-established fluorescent staining (with Nile Red) to selectively target only the MPs in amongst other perhaps inorganics (sand) and biological matter, thus speeding up the analysis by pinpointing which particles are MPs for O-PTIR analysis.

(IR-09.4) Automated Particle Analysis Combined with Raman spectroscopy to Study Rutile Geochemistry for Provenance Analysis

Sarah C. Shidler¹, Tim Prusnick², Lucy Grainger², Achim Hermann³; ¹*Renishaw Inc.*,
²*Renishaw Inc.*, ³*Louisiana State University*

Raman spectroscopy of sedimentary grains shows potential for use in provenance analysis

Raman spectroscopy for the study of Rutile geochemistry shows potential for use in provenance analysis, characterizing sedimentary samples and allowing for the determination of potential source areas that are not possible with traditional methods. This method relies on the identification of TiO₂ mineral polymorphs and other minerals that are present. Raman spectroscopy is ideal for mineral samples, providing clear chemical identification of common minerals and their polymorphs. The non-contact, non-destructive technique preserves the sample for additional analysis methods. In this study, heavy mineral separates were embedded in 1" epoxy mounts with the mineral surfaces exposed and polished. The 80-300 micron sediment grains were analyzed using confocal Raman spectroscopy. Targeted particle analysis allowed for the analysis of grains of interest without analysis of the surrounding epoxy. The Raman results were combined with other analytical techniques to provide a more complete understanding of the sedimentary grains for provenance analysis.

(IR-09.5) Fluorescence Rejection and Improved Identification of Raw Materials and Unknowns with a 785nm Raman System

Elena Hagemann¹, Adam J. Hopkins¹, Naimish Sardesai¹; ¹*Metrohm USA*

Statistically demonstrate the enhanced performance of a 785nm Raman system for identifying highly fluorescent materials

Handheld Raman spectroscopy is used around the globe for identification of unknowns, from trafficked narcotics to raw materials in production processes. Historically, most handheld Raman systems operated with 785 nm laser excitation, the technology of which was miniaturized during the telecom revolution of the 1990s. However, wider and more successful deployment of 785 nm Raman is limited by fluorescence interference in the spectrum. As a result, both industry and government agencies are turning to 1064 nm Raman. 1064 nm Raman has its own drawbacks; including instrument size, component cost, and reduced Raman signal that requires higher laser power and/or longer integration times during sampling.

We have optimized 785 nm Raman with patented fluorescence-rejection technology- XTR®. XTR technology extracts Raman data from the fluorescence background to give a vector of pure Raman data. This eliminates curve fitting artifacts and improves spectral matching for material ID. In this study, we compare traditional 785 nm Raman, 1064 nm Raman, and XTR on challenging samples such as black polypropylene, folic acid, and microcrystalline cellulose to demonstrate the effect of XTR in the Raman spectrum. We evaluate samples according to ROC curve methodology against large analyte

libraries and compare performance using key indicators such as signal-to-noise ratios and measurement time.

22MASS02: Advances in Novel Mass-Spectral Imaging

Chair: Jacob Shelley

(MASS-02.1) Fast Imaging of Polymers Via Laser-Assisted Micro-Pyrolysis Flowing Atmospheric Pressure Afterglow High-Resolution Mass Spectrometry

Dong Zhang¹, Gerardo Gamez¹; ¹*Texas Tech University*

Synthetic polymers and biopolymers are widely used in daily life. Polymer chemical characterization has been of critical importance due to the rapid growth of polymer manufacturing industry. Several analytical techniques, such as nuclear magnetic resonance (NMR) and Fourier-transform infrared (FTIR) spectroscopy have been developed for polymer characterization. However, they are unable to provide exact information on the substructures and functional groups present in polymers and often miss less abundant components. Mass spectrometry (MS) has emerged as a powerful tool for polymer characterization since it can provide chemical information such as repeating unit, end groups, and additives. Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) provides rich chemical information but the chromatography separation time is time-consuming and loses any spatial information within the sample.

Ambient mass spectrometry (AMS) allows direct sample desorption and ionization with minimal-to-no sample preparation. The flowing atmospheric pressure afterglow (FAPA) is a plasma-based AMS source featuring no solvent requirements, and it possess the ability to ionize analytes with a wide polarity range. When combined with pyrolysis techniques, FAPA AMS can be a powerful tool for polymer characterization. Mass spectrometry imaging (MSI) is a powerful characterization tool for heterogeneous and multilayer polymers materials, which allows studying surface defects, aging distribution, phase separation, among others. Herein, a diode laser-assisted micro-pyrolysis (LAMP) technique is demonstrated and coupled with FAPA high resolution mass spectrometry (HRMS) for fast polymer characterization and imaging by taking advantage of the superior spatial resolution provided by the laser spot size. LAMP implements laser power modulation in *real-time* to achieve a varying temperature program, akin to typical pyrolysis strategies, to achieve thermal separation of polymer additives and different pyrolysis products. Characterization of homopolymers, copolymers, polymer blends, as well as lignocellulosic biomass were studied with the aid of data interpretation and visualization tools such as Kendrick mass defect (KMD) plots, van Krevelen plots, and principal component analysis (PCA). Furthermore, MSI of complex polymer samples in both lateral and depth dimensions will be presented.

(MASS-02.2) Parallel Elemental and Molecular Chemical Imaging with Tandem Laser-Ablation Mass Spectrometry and Laser-Induced Breakdown Spectroscopy

Jacob T. Shelley¹, Jacob T. Shelley¹, Sunil Badal, Montwaun D. Young¹, Justin Park, Julia Danischewski; ¹*Rensselaer Polytechnic Institute*

Recent advances in analytical instrumentation and data capture/processing have led to the ability to generate comprehensive chemical maps (or “images”) of solid samples with high spatial resolution. Unambiguous identification of species within samples necessarily requires multiple analytical approaches performed on the same sample often referred to as ‘multimodal chemical imaging.’ While tandem imaging methods provide a wealth of information, they often suffer from weak sensitivity, poor selectivity, or compromised spatial resolution as dictated by the spectroscopic method employed. Greater success in comprehensive chemical imaging has been achieved with mass-spectrometry-based instruments, due to the excellent sensitivity and selectivity. However, mass spectrometry is inherently a destructive technique. Therefore, it would be most advantageous to obtain orthogonal (i.e. complementary) analytical information concurrently with mass-spectral image acquisition. Here, we present a high-spatial-resolution (~10 μm) multimodal imaging approach capable of providing molecular and elemental information from the exact same spatial location (i.e. each pixel).

This dual imaging approach is achieved through laser ablation of samples at atmospheric pressure followed by simultaneous mass-spectrometric and optical-emission measurements. Aerosolized particles from the laser-ablation event were swept with a gas stream to a plasma-based molecular ionization source and, subsequently, mass-analyzed with an Orbitrap mass analyzer. At the same time, atomic emission from the laser-induced plasma (LIP) formed during the ablation event was recorded with a fiber-coupled optical spectrometer to provide elemental information on the laser-sampled area. By scanning the laser across the sample, followed by appropriate data processing, dual atomic and molecular chemical images were generated. Approaches to obtain multimodal atomic and molecular images, as well as the data processing needed to generate and compare chemical images will be presented. Finally, approaches to enable quantitative information for both molecular and atomic analyses will be discussed.

(MASS-02.3) High-throughput Analysis of Leaded and Non-toxic Inorganic Gunshot Residue by spICP-TOFMS

Sarah E. Szakas¹, Korina L. Menking-Hoggatt², Alexander Gundlach-Graham¹, Tatiana Trejos²; ¹*Iowa State University*, ²*West Virginia University*

Elemental characterization and discrimination of inorganic gunshot residue from two ammunition primer classes by spICP-TOFMS.

Single particle inductively coupled plasma time-of-flight mass spectrometry (spICP-TOFMS) has been adapted for forensic analysis of reference inorganic gunshot residue (IGSR) particles.¹ IGSR is a type of trace evidence used to investigate crimes involving firearms. The current standard for analyzing these particles requires examination of both elemental composition and morphology through Scanning Electron Microscopy-Energy

Dispersive X-Ray Spectroscopy (SEM-EDS). While ammunition containing leaded primers form characteristic particles composed of Pb, Ba, and Sb, the use of non-toxic (lead free) primers has increased in recent years, and these particles can have vastly different major elemental compositions. Standard SEM-EDS analyses of IGSR are not optimized for the analysis of non-toxic primer particles, only examine particles above ~1 µm in diameter, and have long analysis times (hours).

spICP-TOFMS offers high-throughput, high sensitivity, and quantitative multi-elemental characterization of individual particles. As applied to IGSR samples, spICP-TOFMS allows for examination of elemental mass profiles, especially of lesser-evaluated non-toxic primer particles, and quantification of variations in particle composition and particle number concentrations. Total analysis time for each sample is less than ten minutes.

IGSR reference samples from leaded Winchester and unleaded Inceptor ammunition were analyzed by both SEM-EDS and spICP-TOFMS. Trends in elemental composition, mass ratios, particle number concentrations, and estimated particle sizes are compared directly. With spICP-TOFMS, up to an order of magnitude more particles per mL are measured compared to that recorded by SEM-EDS. These additional particles are generally smaller than 1 µm in diameter. spICP-TOFMS analysis demonstrates that IGSR particles from the Winchester ammunition contain significant quantities of Cu in addition to Pb, Ba, and Sb; IGSR particles from the Inceptor ammunition consist majorly of Ti and Zn. The compositional differences between the ammunition types enables rapid differentiation of the IGSR types by spICP-TOFMS. This method aims to provide the forensic community with statistically significant data to help improve and inform detection criteria for both leaded and non-toxic IGSR.

1. Menking-Hoggatt, K. et. al., *Talanta* **2021**, 225, 121984.

(MASS-02.4) Fingerprint Beyond the Ridge Detail: Chemical Analysis of Drugs and Toxic Metals in Fingermarks using Magnetic Carbon Nanoparticles and Mass Spectrometry

Mohamed O. Amin¹, Entesar Alhetlani¹, Simona Francese²; ¹*Kuwait University*, ²*Sheffield Hallam University*

Chemical analysis of fingerprint using mass spectrometry can reveal information pertaining to the donor's lifestyle.

For decades, the analysis of fingerprint (FP) has been used as the primary biometric mean of human identification. In parallel the chemical analysis of latent fingermark (LFM), “touch chemistry” offers additional support to forensic examination thus, the development in analytical detection of the FM is crucial. In this regard, we propose a facile approach to synthesize magnetic carbon nanoparticles (MCNPs) from candle soot for extraction and detection of endogenous (e.g. fatty acids, squalene and triacylglycerols) and exogenous FM components including antihistamine, β -blocker, antibiotic drugs and lead. The influence of temperature on the stability of the exogenous substances in the FM revealed the drugs' instability at high temperatures and they undergo different degrees of degradation whereas lead was more resilient to thermal stress. The detection of triprolidine, metoprolol and sulfamethoxazole from pharmaceutical tablets in FM was achieved by gently touching the tablet powder. The LOD values of the drugs in the tablet forms were in the range of 50-750 ng mL⁻¹ and their recovery rates were 91.17-120.86%. Finally, to create a genuine casework scenario, contaminated FM was deposited on glass and metal surfaces then subjected to SALDI-MS analysis using MCNPs as sorbent. Substrate control experiments revealed that the glass surface exhibiting some background signal, however, satisfactory extraction efficiency of FM components on both surfaces was obtained using the MCNPs. Thus, the present study provides exciting opportunities for the use of MCNPs as new SALDI-MS substrate for both extraction and detection of FM components providing more information pertaining to the donor's lifestyle.

(MASS-02.5) Development and Characterization of Low-Cost Liquid Sample Introduction System for ICP-MS

Tristen Taylor¹, Alexander Gundlach-Graham¹; ¹*Iowa State University*

We developed a new sample-introduction approach for ICP-MS based on capillary vibrating sharp-edge spray ionization.

In this research, we present the use of capillary vibrating sharp-edge spray ionization (cVSSI) as a nebulization source for inductively coupled plasma mass spectrometry.¹ Each cVSSI nebulization device is about 1% of the price of a conventional pneumatic nebulizer.

When using a conventional nebulizer and spray chamber all gas flows for sample transport typically come from the nebulization gas, leaving little room for optimization of transport efficiency. In contrast, cVSSI is a completely gas free nebulization source, which decouples the mechanisms of aerosol creation and transport. This decoupling has allowed us to reimagine the traditional spray chamber. Chambers are 3D modeled and printed using fused deposition modeling. This method of production allows for quick prototyping and testing of chamber designs. Nebulized aerosol is transported with an on-axis carrier gas and curtain

gas in the chamber. For either gas, argon can be replaced with helium to facilitate droplet desolvation, allowing for the introduction of larger droplets into the ICP and increased sensitivity. Additionally, the frequency and voltage inputs of the cVSSI device can be tuned to optimize nebulized aerosol characteristics.

Using ICP-TOFMS, we have examined the reproducibility between nebulization devices for this system as well as compared transmission efficiency and stability to that of a pneumatic nebulizer and cyclonic spray chamber at low flow rates (< 20 $\mu\text{L}/\text{min}$). Based on successful proof-of-principle studies, we are using computational fluid dynamics (CFD) to characterize and optimize the chamber. Experimental results from our sample introduction system and chamber optimization results from CFD simulations will be presented. We will demonstrate that cVSSI is a promising sample introduction approach for ICP-MS that is a much less expensive alternative to traditional sample introduction systems.

1. N. Ranganathan. et.al. *Am Soc Mass Spectrom*, **2019**, 30, 824-831.

22PMA03: SERS for Drug Discovery

Chair: Colin Campbell

Co-Chair: Lamyaa Almeahadi

(PMA-03.1) **Alkyne-tag SERS imaging for drug detection in living cells**

Katsumasa Fujita¹; ¹*Osaka University*

Alkyne tag Raman imaging is a promising technique for observing small molecules in living cells. The advantage of using Raman tags is that the perturbation to the target molecule is small compared to fluorescent probes. However, the small scattering cross section of Raman imaging makes it difficult to detect low concentration molecules, which widely limits its practical application. We have attempted to use SERS with gold nanoparticles introduced into living cells to enhance Raman signals from alkyne tags for time-lapse monitoring of drug uptake into cells. Using alkyne-tagged Z-AOMK (an inhibitor of protease cathepsin B), 3D time-lapse SERS imaging was performed by applying the alkyne-tagged drug on live macrophages incubated with gold nanoparticles before the experiment. As a result, Raman signals from alkyne in the cell-silenced region were successfully detected, as well as their temporal changes with the uptake of the drug into the cell. Time-lapse 3D SERS imaging allows us to monitor drug uptake in 3D and estimate the rate of drug uptake by counting the SERS spots of the alkyne tag. The number of SERS spots and their temporal increase shows a trend that increases in proportion to the concentration of drug applied to the cells. We have also applied the Alkyne SERS imaging technique to confirm the temperature dependence of drug uptake, showing that drug molecules are taken up passively across the cell membrane.

(PMA-03.2) **Surface Enhanced Raman Scattering to Assess Sub-Cellular Nanoparticle Delivery**

Brian Scarpitti¹, Zac D. Schultz¹, Sanjun Fan¹; ¹*The Ohio State University*

Plasmonic nanoparticles (NPs) are of interest in drug development for delivery of small molecules or as therapeutic agents (such as in Photodynamic therapy). This interest arises in part from the pharmacokinetic properties of nanoparticles relative to free small-molecule drugs. To realize more effective delivery, nanoparticles are typically decorated with targeting molecules. The efficacy of these targeting molecules can be assessed by functional assays; however, a technique that provides non-invasive, rapid assessment of nanoparticle accumulation in living tissue could improve efficacy and reduce toxicity. Surface enhanced Raman scattering (SERS) has been used to assess delivery of plasmonic nanoparticles in cell culture and some animal models. SERS can characterize particle delivery to outer membrane receptors (such as integrins) that are overexpressed in some cancer cells, enabling tissue selective nanoparticle delivery. To increase the number of drug targets accessible to nanoparticle therapeutics, efficient nanoparticle delivery to the cytosol of cells is needed. We are working toward quantifying nanoparticle delivery in cell culture, with a particular interest in monitoring endosomal escape of nanoparticles. Here we report on efforts toward in vitro NP detection to address some outstanding challenges including: 1) achieving single nanoparticle detection in a dynamic sample, 2) quantifying SERS intensity fluctuations to count NPs in cells, and 3) assessing the chemical stability of SERS tags in cell culture. Addressing these challenges will increase the utility of SERS to rapidly screen cytosolic drug delivery systems, and advance the use of NPs in therapeutic applications.

(PMA-03.3) Machine Learning Enabled SERS: Applications and Potential for Medical Diagnostics and Drug Discovery

Joy Q. Li¹, Joy Q. Li¹, Tuan Vo-Dinh¹; ¹*Duke University School of Medicine*

Surface-enhanced Raman spectroscopy (SERS) is used in a wide array of diagnostic applications due to its high sensitivity and narrow spectral features which allow high degrees of multiplexed analysis. Machine learning (ML) is a rapidly developing and popular tool for SERS spectral analysis. Deep learning holds promise in aiding SERS analysis with the ability to learn complex patterns from data. We compared the performances of several traditional and ML approaches including spectral decomposition and convolutional neural network (CNN) for analyzing a multiplexed mixture of 7 SERS-active nanoparticles loaded with different dyes for mRNA biomarker detection. CNN was then used to analyze SERS spectra from a single-plex, point-of-care (POC) assay detecting an mRNA biomarker for head and neck cancer in 20 samples. ML and deep learning are also rapidly emerging tools for many stages of drug discovery applications. These ML methods have great potential in facilitating target validation, biomarker identification, and analysis of clinical trial data. The challenges in ML applications lie in limited interpretability and dataset generation. We will provide an overview of various ML applications to drug discovery and their challenges and limitations.

(PMA-03.4) Label-Free SERS for Drug Discovery: Hit Identification

Lamyaa M. Almeahadi¹, Vibhav A. Valsangkar¹, Ken Halvorsen¹, Qiang Zhang¹, Jia Sheng¹, Igor K. Lednev¹; ¹*University at Albany, State University of New York*

The drug discovery field relies on sensitive, specific, and rapid methods to identify hits. The detection and characterization of molecular binding events between potential drugs and their targets is a key step in the drug discovery process. Surface-enhanced Raman spectroscopy (SERS) is an emerging tool for detecting such molecular interactions. Current methods used for drug discovery would profit from SERS's high sensitivity and label-free capability, thus limiting laborious steps and resource-consuming needs.

Our platform is used to detect binding events of peptide ligands to targeted RNA repeats at a nanomolar concentration. The binding trends found using SERS detection correlated with the binding affinity of different ligands used in this study. The differentiation between binding and non-binding ligands was possible via visual inspection and statistical analysis

(PMA-03.5) A new SERS approach to monitor responses to therapy in live 3D tissue models.

Colin J. Campbell¹, William Skinner¹, Norbert Radacsi¹, Robert Gray¹, Michael Chung¹, Nicola Robinson¹, Gareth Hardisty¹; ¹*University of Edinburgh*

We have developed novel SERS substrates that can be incorporated within live 3D tissue models.

3D tissue models are increasingly important in the animal-free development of treatments for disease. In many cases, patient-derived 3D tissue models exhibit disease-like characteristics in a more "human" way than available animal models. A challenge in the use of 3D tissue models is the real-time monitoring of their response to therapies. Our approach has been to monitor the metabolic activity of cells in 3D by measuring local pH differences. To make these measurements we have developed new SERS substrates that can be easily integrated into a live 3D tissue model. These SERS substrates are both micron-sized SERS microspheres that can make measurements at epithelial interfaces and SERS meshes that can be used as a scaffold to support cell growth and make measurements with unparalleled spatial resolution. Our substrates exhibit strong reproducible signal intensity and are broadly accessible since they require no clean-room facilities to fabricate. I will show data collected from liver cancer models and cystic fibrosis models.

22RAM05: IRDG Raman

Chair: Karen Faulds

(RAM-05.1) Radiation Response Monitoring in Biological Systems Using Raman Spectroscopy and Machine Learning Techniques

Andrew Jirasek¹, Andrew Jirasek¹, Kirsty Milligan¹, Ramie Ali-Adeeb¹, Phillip Shreeves¹, Juanita Crook², Alexandre Brolo³, Julian Lum⁴, Jeffrey Andrews¹; ¹*University of British Columbia*, ²*BC Cancer, Kelowna, Canada*, ³*University of Victoria*, ⁴*BC Cancer - Victoria*

Radiation therapy (RT) employs high energy photon or particle beams to kill tumour cells. Ongoing technical advances in RT now enable exquisite dose sculpting to target disease sites while concomitantly sparing healthy tissue. Both external beam and internal, or

brachytherapy, techniques are routinely employed in radiotherapy, and the choice of technique depends upon a range of clinical factors including disease site and local expertise.

Despite these advances, the prescribed doses used in RT remain derived from past population averages and clinical experience. Currently no biochemical markers of disease response are measured and used pro-actively in tuning radiation dose to individual patient response or inherent radio sensitivity. An array of assays for this end are currently under development and range from imaging based through to genomic-based marker identification.

We here employ Raman spectroscopy as a prospective tool for the identification of biochemical response to radiation. We have developed an analytical framework, termed Group and Basis-Restricted Non-Negative Matrix Factorization (GBR-NMF), allowing for Raman spectra to be decomposed into individual, user-input, biochemical bases spectra and their corresponding weights, or contributions. GBR-NMF then allows for tracking of individual biochemical dynamics as a function of cellular and tissue radiation response. Furthermore, using a Random Forest (RF) classifier we are able to identify the largest biochemical contributors to spectral dynamics.

We employ our Raman-based analytical framework to show that we can reconstruct biochemical dynamics previously observed in irradiated cellular studies. We further extend our work to the study of patient prostate biopsy samples acquired pre and post 13.5 Gy single fraction high dose rate brachytherapy treatment. We show that Raman spectra acquired on pre-treatment biopsies can be used to predict pre treatment Gleason and CAPRA (Cancer of the Prostate Risk Assessment) scores with 66% and 70% accuracy, respectively, and post-treatment Ki67 (tumour proliferation) with 85% accuracy.

In summary, our work highlights the capabilities of Raman spectroscopy as a prospective tool for measuring biochemical response to radiotherapy treatments. The long range aims of the technique centre around predictive and treatment monitoring applications in radiation therapy.

(RAM-05.2) Effect of Laser Power and Exposure Time on Live Cell Raman Measurements.

Alison Hobro¹, Kota Koike¹, Takeshi Sugiyama¹, Nicolas Pavillon¹, Takayuki Umakoshi¹, Prabhat Verma¹, Katsumasa Fujita¹, Nicholas I. Smith¹; ¹*Osaka University*

Exploring the trade-off between measurement time/power, Raman spectral quality and potential for inducing cellular changes.

Raman spectroscopic measurements of live cells can provide insights into the nature and, for imaging modes, distribution of molecules within the cells, and how these change in response to external stimuli or stresses. However, it is not always clear what the most effective measurement protocol might be for investigating a particular biological problem – the most obvious trade-off being the ability to collect sufficiently detailed Raman spectra

without damaging the sample. Here we have used four different measurement modes (1) standard imaging, (2) line skip imaging, where a reduced number of lines are recorded producing a sub-sampled image, (3) standard single point measurements and (4) spatially averaged single point measurements, where the sample (or laser point) is scanned during the measurement to provide a single point spectrum obtained from a larger area. In each case, we have assessed how different laser power and different exposure times affect the nature/quality of the Raman signal generated, as determined by the potential for the measured spectra to distinguish between two different cell lines. In addition, sequential measurements performed on each cell enable us to assess the impact of a Raman measurement on the measured cell and to detail the nature of the laser-induced cell changes occurring for each measurement mode and laser power/exposure time.

(RAM-05.3) Designing Assemblies of Nano-Gold for Improved Raman Sensing

Priyanka Dey¹; ¹*Teesside University*

Gold nanostructures and surface enhanced Raman scattering (SERS) has been truly synergistic and critical for biomedical Raman. My interest has been to explore ways to control nanoparticle (NP) interactions in colloid and exploit the coupled electric field i.e., “hot-spots” for in vivo applications. The coupled electric field observed when two or more NPs are close to each other results in strengthening the plasmon coupling further at the NP-NP junctions or hot-spots. The Raman signal of any molecule (here, label) that sits at these hot-spots experience tremendous SERS enhancements (10^3 - 10^{14} times). The design of plasmonic nano-assemblies are thus critical. Salts and small organic molecules have often been employed resulting in random assembly formation with low colloidal stability. Thus, our approach is to employ sub-10 nm branched polymers as linking molecules between NPs to form sub-100 nm gold nano-assemblies, allowing controlled assembly formation with colloidal stability. In the presentation, I will discuss the key approaches to enable the design of gold nano-assemblies into different morphologies. For example, dimers, nanochains, nanobranches, core-satellite, and the patented core multi-tentacles. Specific nano-assembly morphologies allow control of the NP-NP gap, hot-spot density, and intensity, positioning of the SERS molecule at the hot-spot. They thereby ensure the SERS enhancement efficiency and reproducibility of colloidal SERS sensors. Thus, by manipulating the NP-NP junction properties and SERS amplification potential, we have shown that these colloidal nano-assemblies can significantly increase detection depths from within animal tissues using spatially offset Raman, when compared to similar single gold nanoparticles. Additionally, enhanced plasmonic heating is featured by nano-assemblies, enabling photothermal therapy for cancer. Thus, such custom-designed gold nano-assemblies are gaining limelight as cancer nanotheranostic agents.

(RAM-05.4) Brillouin Microscopy to Probe Viscoelastic Properties of Tissues in Health and Disease

Michelle Bailey¹, Francesca Palombo¹; ¹*University of Exeter*

Brillouin Microscopy (BM) is an all-optical technique, providing information on micromechanics through the scattering of light from acoustic waves, or phonons. The mechanical properties within the biological environment are crucial to the health and vitality of living systems, and alterations in mechanics can thereby indicate disease. Biological applications of BM have ranged from the measurement of live cells and organisms, to tissues and fibrous proteins, demonstrating potential for diagnosis of pathology and characterisation of biomechanics.

In this talk, the application of Brillouin microscopy to probe the viscoelastic properties of biologically relevant samples will be discussed. Initially, tissue-mimicking hydrogels made of gelatin (denatured type-I collagen), with tuneable physical and mechanical properties, were studied to elucidate the origin of BM signals. Gelatin hydrogels were studied over a range of concentrations, and effects of temperature and water content were investigated. Most striking was the observation of a glass transition controlled by change in polymer concentration, denoted by a sigmoidal evolution of the Brillouin frequency shift and a maximum in linewidth. This corresponds to a dramatic slowdown of the structural relaxation process, ubiquitous to colloidal systems and, for the first time here, observed with BM in biological-based systems. Further to this, BM measurements on more complex biological systems will be discussed, including the use of BM to probe cellular mechanics in the context of cancer. The introduction of Raman spectroscopy as a correlative technique will also be presented. This enables the chemical composition and density to be determined simultaneously to the mechanical properties probed by Brillouin spectroscopy, thus providing a comprehensive assessment of the sample. These results have shown Brillouin microscopy to give a unique description of the viscoelastic properties across a wide range of biologically relevant physical states, from the highly hydrated to the solid-like phase, and the transition between the two, as well as providing a new contrast mechanism in mechanobiology.

(RAM-05.5) Ratiometric SESORS Imaging and Detection: Towards Locating Nanotags at Depth in 3D

Matthew E. Berry¹, Samantha M. McCabe¹, Sian Sloan-Dennison¹, Stacey Laing², Neil C. Shand³, Duncan Graham¹, Karen Faulds¹; ¹*The University of Strathclyde*, ²*University of Strathclyde*, ³*The Defence Science and Technology Laboratory (DSTL)*

In this work we address a fundamental question crucial to understanding SESORS imaging and implementing it in a clinical setting for *in vivo* diagnostic purposes in the future, namely can SESORS be used to give the physical location of SERS active nanotags through tissue? We report the effects of the spatial offset magnitude and geometry in locating nanotags through tissue and outline experimental techniques to allow for the correct interpretation of SESORS images to ascertain nanotag location in the 2-dimensional *x, y*-imaging plane at depth. More specifically, we present the effect of ‘linear offset induced drag’ which refers to a spatial distortion in SESORS images caused by the magnitude and direction of the linear offset and highlight the need for an annular SORS optical geometry during imaging to neutralise these asymmetric effects. Additionally, building on these principles, we introduce the concept of ‘ratiometric SESORS imaging’ for the location of buried inclusions in 3-

dimensions. We tested the robustness of our approach, which utilises the relationship between spatial offset magnitude, the probed depth and ratiometric analysis of the nanotag and tissue Raman intensities, to image and spatially discriminate between two distinct nanotag flavours buried at different depths within a 3-dimensional model for the first time. In an alternative methodology, we introduce a proof-of-concept calibration technique for the prediction of the depth of different flavours of SERS active nanotags embedded within porcine tissue using the same ratiometric analysis technique, which can also be termed as the “relative contribution” of the nanotags to through tissue SORS measurements. As the tissue barrier thickness is increased incrementally, an accurate log-linear calibration can be developed that correlates the Raman response of the multi-layered system with the depth of the buried nanotags. This method differs from others in that it is the first to exploit the exponential decay in the relative contribution of the nanotags in SORS spectra with increasing depth and also to utilise PCA for defining the calibration limit. Overall, our work represents a significant step forward in the ability to determine the location of multiple vibrational fingerprints at depth in a clinical setting.

22RAM12: Raman Spectroscopy for Security and Forensics Purposes

Chair: Igor Lednev

Co-Chair: Sonivette Colón-Rodríguez

(RAM-12.1) Understanding how Matrix Composition Influences the Detection of Drugs using Raman and SERS

Amanda J. Haes¹, Amanda J. Haes¹; ¹*University of Iowa*

Solvent and ion choice can promote or limit the sensitive detection of small molecules using plasmonic nanoparticles and surface-enhanced Raman scattering (SERS). This is, in part, due to the high solvent/ion concentration relative to that of the analyte as well as inherent interactions between molecules, nanostructures, and/or solvent. Herein, SERS detection of aspirin and other small drug molecules is evaluated as a function of co-solvent and ion composition. By using gold nanostars that are electromagnetically stable, both low and high abundance molecule-solvent-nanoparticle complexes are revealed through changes in vibrational signatures. By better understanding how solvent contributes to competing intermolecular interactions, ultrasensitive detection of small molecules can be expanded.

(RAM-12.2) The classification of Raman scattering patterns using wavelet transform and transfer learning

Jorn Yu¹, Ting-Yu Huang¹; ¹*Sam Houston State University*

A new intelligent analytical platform for field identification and classification of materials using a Raman spectrometer and transfer learning will be reported in this presentation. A hand-held Raman spectrometer was utilized to collect Raman spectra of reference samples. Then, the Raman scattering pattern was converted into image presentations by continuous wavelet transformation (CWT) to facilitate artificial intelligence (AI) using the transfer learning technique. GoogLeNet, a pre-trained convolutional neural network (CNN), was evaluated to train the classification model. Several conventional machine learning algorithms were also compared to assess the performance of our new approach. The data

processing and workflow to develop the AI classifiers for ignitable liquids and polymers will be discussed as examples in this talk. The experimental results indicated that the pre-trained CNN model developed by our new data workflow outperformed other models in several performance benchmarks. We believe the wavelet transform process combined with transfer learning will accelerate AI development using hand-held Raman spectrometers for various field applications, such as forensics, quality control, and homeland security.

(RAM-12.4) Utilizing Raman Spectroscopy to determine the Time Since Deposition of Menstrual Blood Stains

Alexis R. Weber¹, Anna Wójtowicz², Igor K. Lednev¹; ¹*University at Albany, State University of New York*, ²*Jagiellonian University*

This work shows that Raman Spectroscopy can analyze menstrual and peripheral blood with similar accuracy.

Blood traces are commonly found at crime scenes and can provide substantial information about the event that occurred, and individuals involved. There are two main types of bloodstains, peripheral blood and menstrual blood. There are several techniques that can be used to discriminate between peripheral and menstrual, but once identified it would be ideal to obtain more information from the sample. Determining the time of crime is an important goal for crime scene investigations, which can be achieved by estimating the time since deposition (TSD) of bloodstains. If crime scenes contain multiple sets of bloodstains, the calculated TSD should allow for the selection of bloodstains relevant to the crime; and therefore, reduce the number of samples which should be collected, documented, and processed.

Vibrational spectroscopy paired with chemometrics has shown provide reliable, rapid, and non-destructive methodologies to determine the TSD of bloodstains. However, all the work that has been conducted thus far have been on peripheral bloodstains. Menstrual blood is also commonly found at bloodstains and can be present as a result of tissue damage from an assault or a natural cause. Determining the TSD of the menstrual bloodstain can help to reconstruct the event and differentiate between stains that are relevant vs those that are extraneous. At a crime scene both peripheral and menstrual bloodstains can be present. As such, it is pertinent to understand if the capabilities of Raman spectroscopy are able to estimate the TSD of menstrual bloodstain as well as this method does for peripheral bloodstains. To fill this gap in the research, a novel method for the prediction of TSD of menstrual bloodstains, based on Raman spectroscopy was created.

(RAM-12.5) Latest Advances in Handheld Raman Usability and Performance

Luisa T. Profeta¹, Brian L. Bures¹, Huwei Tan¹, Adam J. Maines¹, Kurt R. Bistany¹, Stefan R. Lukow¹, Michael D. Hargreaves¹; ¹*Rigaku Analytical Devices*

Pushing the boundaries of refining handheld instrument algorithms and processing for unknown chemical identification.

Raman devices have been deployed in chemical identification applications for well over a decade. Since their inception, they have become more advanced, following technology trends in other industries. Advanced variants utilize new and upcoming technologies to differentiate from the increasingly saturated market, with improvements to lasers, gratings, detectors, and further miniaturization. This presentation will detail the latest graphical user interface (GUI) updates for the Rigaku CQL instrument, which focus on reducing customer cognitive burden and providing the most critical information for those making critical decisions in law enforcement, hazmat or SSE applications. The new GUI helps to provide clear intuitive usability, especially for infrequent users of equipment, modelled after common consumer interfaces. Additionally, this presentation will examine the latest data collection updates, including our optimization of preserving weaker Raman signals amid noisier backgrounds typically encountered by field users (e.g., sunlight) in addition to tackling the challenges of collecting Raman signals of naturally fluorescent materials. Detailed are our continued improvements of the chemical identification algorithm performance, with decreased false alarms by 2x and increased specificity of mixture analysis. Examples of algorithm changes will be demonstrated with explosives, narcotics and other CBRNE materials of interest.

22SPECIAL06: Regional Academic Research

Chair: Pietro Strobbia

(SPEC-06.1) What Lies Beneath your Elution Peak: Imaging When and Where Analytes Adsorb to Commercial Stationary Phase Particles

Lydia Kisley¹, Lydia Kisley¹, Ricardo Monge Neria; ¹*Case Western Reserve University*

Studying separations from the bottom-up — one molecule at a time — can identify rare events that lead to the failure of challenging, high-purity separations that are hidden in conventional experiments (chromatography, isotherms, breakthrough curves, *etc.*). Single-molecule fluorescence microscopy, an optical imaging technique, resolves spatiotemporal nanoscale dynamics that occur in separations. Millisecond time resolutions and sub-diffraction limited, 3D spatial resolutions at ~10 nm are achieved *in situ*. Single-molecule fluorescence microscopy has previously detected and quantified the causes of common problems in reverse-phase, normal-phase, ion-exchange, and membrane separations, yet prior sample conditions were obtained from overly-simplified model substrates and low concentrations of a single analyte. Here, we expand single molecule microscopy to quantifying analyte dynamics on commercial chiral stationary phase particles. We map the adsorption sites in 3D over at ~10 nm resolutions showing rhodamine 6g analytes only adsorb to the edges but do not enter the interior of the particle of fully porous particles. We quantify at adsorption rates, desorption rates, and free energy at individual binding sites,

showing intra- and interparticle heterogeneity. Our results demonstrate how single molecule microscopy can reveal the underlying adsorption-desorption phenomena that lead to peak broadening in bulk scale separations.

(SPEC-06.2) Super resolution Spectral SERS imaging

Zac D. Schultz¹; ¹*The Ohio State University*

Advances in nanotechnology enable the detection of trace molecules from the enhanced Raman signal generated at the surface of plasmonic nanoparticle. We have developed technology to enable super-resolution imaging of plasmonic nanoparticles, where the fluctuations in the surface enhanced Raman scattering (SERS) signal can be analyzed with localization microscopy techniques to provide nanometer spatial resolution of the emitting molecules location. We have been able to use this approach to increase the spatial resolution of SERS imaging. While we can visualize SERS fluctuations across a wide field of view, the spectral information in these experiments generally requires a second measurement after acquiring the super-resolved image. Recent work now enables the super-resolved SERS image and the corresponding spectrum to be acquired simultaneously. This spectrally resolved SERS imaging provides two spatial, a frequency dimension, and a time dimension, providing increased information characterization of molecules interacting with plasmonic nanoparticles. In this presentation we will discuss the instrumentation, nanoparticles, and data illustrating the imaging of the Raman signal from nanoparticle probes to understand activity in biological and other systems.

(SPEC-06.3) Real Time, Localized Measurement of Self Assembled Monolayer Formation

Ryan White¹, Hope Kumakli; ¹*University of Cincinnati*

Scanning electrochemical cell microscopy (SECCM) is a unique tool that provides localized reactivity measurements at interfaces. We leverage this measurement ability to study the formation of self-assembled monolayer chemistry of thiol-on-gold monolayers. Using SECCM, we monitor the real-time formation of monolayers on polycrystalline gold electrodes. The nm-scaled probe size allows us to measure how the underlying substrate affects monolayer integrity and rate of monolayer formation. More specifically, we can study monolayer formation at single crystal faces as well as boundaries between crystal grains. Monolayer formation is monitored using voltammetry and chronoamperometry of a soluble redox marker. The formation of insulation monolayers impede faradaic electrochemistry and surprisingly leads to a instantaneous drop in current below measurable levels. The time for this drop in current to occur is both adsorbate identity (carbon chain length) and concentration dependent. We believe that this new measurement can provide new insight into their mechanism on self-assembled monolayer formation.

(SPEC-06.4) Circular Dichroism study of supramolecular systems

Angela Mammana¹; ¹*University of Dayton*

Nature is able to overcome the limits of organic synthesis by adopting a specific logic by forming complex supramolecular systems that are created using non-covalent molecular interactions including electrostatic forces, hydrogen bonds, van der Waals forces, and donor-acceptor interactions as “glue” to hold the single molecule building blocks together in a defined structure. Supramolecular chemistry allows for complex structures to be created without the use of energetically costly covalent bonds and takes advantage of the high degree of reversibility inherent in intermolecular interactions. A circular dichroism (CD) and UV-Vis spectroscopic study of the induced chirality of various molecular systems will be presented. The effect of pH, time and chiral template on the assembly of achiral porphyrins and molecular photo-switches into chiral supramolecular structures will be presented.

(SPEC-06.5) Engineering CRISPR-Cas Biosensors for Environmental and Infectious Disease Monitoring using Nucleic Acid Nanotechnology

Kevin Yehl¹; ¹*Miami University*

Cas12-based biosensors are showing tremendous potential for rapid and portable nucleic acid detection. This is enabled by Cas12's unique trans-cleavage activity, where upon target nucleic acid recognition, Cas12 becomes activated and cleaves ssDNA reporter substrate for enzymatic signal amplification. However, first-generation Cas12-based biosensors require DNA amplification prior to detection and are limited in analytical scope to only detecting nucleic acids. Here, I will discuss strategies employing DNA nanotechnology to improve the sensitivity, speed of detection, and analyte scope for Cas12 biosensors. Such technology will prove extremely useful for tracking and containing infectious disease outbreaks and expanding Cas12's utility to tracking environmental contaminants.

22SPECIAL08: Spectrochimica Acta B - Award Session

Chair: Alessandro De Giacomo

(SPEC-08.1) A Critical Evaluation Of Liquid-Based Glow Discharge Atomic Emission Spectrometry: Future Star... Or Bust?

Steven J. Ray¹; ¹*The State University of New York at Buffalo*

A new generation of liquid-based glow discharge plasmas are being developed for atomic spectrometry which hold a number of significant advantages over current technologies. One example, the solution-cathode glow discharge (SCGD) is a simple, low power (80W), portable plasma sustained in ambient atmosphere directly upon a sample solution that is being developed for atomic emission spectrometry (AES). The sample solution is directly sampled by the SCGD plasma, with analyte entering the plasma directly to be excited. The resulting emission spectra are mainly composed of neutral atomic lines. No purified support gas is required and the plasma is primarily composed of air and water vapor. Surprisingly, the SCGD and similar liquid-based glow discharges can often offer limits of detection competitive with established techniques like Inductively-Coupled Plasma Atomic Emission Spectrometry (ICP-AES). These capabilities coupled with the minimal operating requirements of the plasma have made the SCGD-AES experiment an attractive candidate for on-line, continuous, or in-field applications. Here, we will critically examine the current

capabilities of SCGD-AES and similar liquid-based glow discharge experiments. Current analytical capabilities will be reviewed and compared with extant methods, and recent analytical methods and strategies will also be described. Finally, the place of these liquid-based glow discharge experiments among the field of analytical spectrometry techniques will be considered in order to answer the question: Should liquid-based plasmas like the SCGD be considered as a viable solution for modern atomic spectrometry applications...or are they a bust?

(SPEC-08.2) A Searchable/Filterable Database of Elemental, Doubly Charged, and Polyatomic Ions that Can Cause Spectral Overlaps in Inductively Coupled Plasma-Mass Spectrometry

John W. Olesik¹, Madeleine C. Lomax-Vogt¹, Fang Liu¹; ¹*Ohio State University*

Spectral overlaps are one of the main challenges in ICP-MS. If the signal from ions resulting in a spectral overlap is significant compared to the analyte ion signal at the same m/z , the measured analyte concentration will be inaccurate if the spectral overlap is not accounted for, removed, or avoided. To account for the spectral overlap mathematically, to use a collision/reaction cell to remove the overlap (typically with charge transfer reactions), or avoid the overlap (by using an alternative analyte ion isotope or atom addition reaction to move the analyte ion to a higher m/z), the identity of ion causing the spectral overlap must be known.

Previously available lists of elemental, doubly charged and polyatomic ions were limited. Not all of the ions in those lists were experimentally verified to exist. Some ions that do exist were not included in the lists.

We measured complete mass spectra from 74 “single element” solutions and identified each ion in each mass spectrum. Over 2000 different ions were identified. This often required using a linear combination of element isotope patterns to best match the experimental spectra. More than 50 ions (150 isotopes) not previously described in the literature to our knowledge were identified.

The database can be searched, sorted, or filtered in Excel based on m/z , analyte element, element contained in the ion that could cause a spectral overlap or ion type. Elemental, doubly charged elemental, oxide, hydroxide, hydride, dioxide, trioxide, and doubly charged polyatomic ions are included in the database. We will show examples of using the database to assess potential spectral overlaps in specific sample types.

Future improvements to the database, including ions (such as NaCl^+ , NaS^+ , TiC^+ and CaC^+) produced from solutions that contain one element of interest and a high concentration of another element will be discussed. The current database provides no quantitative measure of the severity of spectral overlaps. An approach to estimate the severity of spectral overlaps will be described.

(SPEC-08.3) A Demonstration of Spatial Heterodyne Spectrometers for Remote LIBS, Raman Spectroscopy, and 1D Imaging

K. Alicia Strange Fessler¹, Stanley M. Angel², Abigail M. Waldron¹, Arelis Colon, J. Chance Carter³; ¹*Savannah River National Laboratory*, ²*The University of South Carolina*, ³*Lawrence Livermore National Laboratory*

Three spatial heterodyne Raman spectrometers, one free standing, the others monolithic, have been used for remote Raman and [LIBS](#) for samples at a distance of 4.5 m and 1D [Raman imaging](#). The wide area measurement capability of the SHS was demonstrated and shown to reduce sample [photodegradation](#) in the case of Raman, using large laser spots on the sample, without loss of signal or decreased spectral resolution. 1D Raman imaging using a free standing SHRS and a monolithic SHRS was demonstrated and shown to provide better signal-to-noise ratio (SNR) spectra for heterogeneous samples than spectra measured without imaging. Improved SNR using 1D imaging is the result of spatial separation of the signal from different areas of the sample, which reduces the contribution of shot noise from stronger scattering sample regions to more weakly scattering adjacent sample regions. For 1D imaging of adjacent samples, within the field-of-view (FOV) of the spectrometer, the SNR improved up to four times, with no loss of spectral resolution or spectral range, and a spatial resolution of 280 μm was demonstrated for samples located at 4.5 m from the spectrometer.

(SPEC-08.4) **Calibration-Free LIBS: What's New After 20 Years?**

Vincenzo Palleschi¹; ¹*CNR, Italy*

The Calibration-Free LIBS (CF-LIBS) method was developed by the Pisa group in 1999 and, since then, it has been widely applied for standardless quantitative analysis of many different materials. The main characteristics of CF-LIBS, besides its capability of doing analytical measurements without standards, is the possibility to overcome the matrix effect, which in LIBS and other spectrochemical techniques forces the use of matrix-matched standards for building univariate or multivariate calibration curves/surfaces. In this communication, we will discuss the evolution of the CF-LIBS technique in the 20+ years since its introduction, describing the methods developed for overcoming the effect of optically thick plasmas and the 'hybrid' uses of CF-LIBS in One-Point Calibration CF-LIBS or in combination with an Artificial Neural Network for speeding-up the calculation on large sets of LIBS spectra. The new 3D-CF-LIBS technique i.e., the use of a 3D Boltzmann plot (the third dimension being the time after the breakdown) in CF-LIBS analysis will be also discussed.

(SPEC-08.5) **Glow Discharge Optical Emission Spectroscopy Ultra-High Throughput Elemental Mapping: Insights into the Underlying Mechanisms via Laser Scattering Techniques**

Gerardo Gamez¹, Kevin Finch¹; ¹*Texas Tech University*

Glow discharge optical emission spectroscopy (GDOES) allows surface elemental mapping when the GD is operated in pulsed-power mode (<1kHz, <10% duty cycle) and at higher pressures (10-30 Torr), with the added advantage of obtaining high-pixel density maps with at least three orders-of-magnitude higher throughput compared to typical techniques.

Nevertheless, the effect of such operating conditions on the plasma parameters is yet to be studied, thus the changes to the GD underlying mechanisms are not well understood.

Laser scattering (LS) plasma-diagnostic techniques (Thomson, Raman, and Rayleigh) have an inherent spatial and temporal resolution, minimal-to-no plasma perturbation, and do not require assumptions of local thermodynamic equilibrium. While the required LS instrumentation can be complex when it comes to their implementation on low-density plasmas, such as in GDOES, they are the method of choice when available, given all their advantages.

Here, a previously described in-house built LS system featuring a transmission-type triple grating spectrograph will be utilized to obtain spatiotemporally resolved maps of electron temperatures/densities, pertaining to a variety of operating conditions commonly implemented during GDOES EM. A new method for the spatial and spectral correction of the spectral imaging system based on measured vs simulated Raman Scattering will be presented. Also, the instrument performance tested with different types of cameras will also be discussed. Finally, the effects of pressure and RF power on the fundamental parameters will be examined as a function of spatial position in the plasma and time along the pulse. These studies will allow obtaining necessary insights into the plasma underlying mechanisms under GDOES EM operating conditions.

22PLEN03: Coblenz Society Clara Craver Award

(PLEN-03.1) Stimulated Raman Scattering Imaging: From Label-free to Metabolic to Super-multiplex and to Single-molecule Imaging

Wei Min¹; ¹*Columbia University*

All molecules consist of chemical bonds, and much can be learned from mapping the spatiotemporal dynamics of these bonds inside cells, tissue and animals. Since its invention in 2008, stimulated Raman scattering (SRS) microscopy has become a powerful modality for imaging chemical bonds with high sensitivity, resolution, speed and specificity. The past dozen years have witnessed the blossoming of SRS microscopy, where advances in both optical instruments and imaging probes have found broad applications in life sciences. Here I will present the exciting development in our group, from label-free imaging to metabolic imaging in animals to super-multiplexed imaging and to single-molecule vibrational imaging.

22PLEN03: NESAS and SAS Lester W. Strock Award

(PLEN-03.2) NESAS and SAS Lester W. Strock Award

Igor B. Gornushkin¹; ¹*BAM Federal Institute for Materials Research and Testing*

In this presentation, I will give a brief overview of my personal experience with laser induced plasma (LIP). I will start from my and colleagues' early works, where we used LIP as an atomic reservoir for laser induced fluorescence (LIF). We applied LIP-LIF for a sensitive detection of trace elements in various materials and demonstrated that under certain conditions the technique can even be used for isotope analysis. Next, I will discuss

the application of LIP spectroscopy, i.e., LIBS, to material identification that nowadays constitutes one of the best applications of this technique. In those early days, we used correlation analysis for spectra processing; it is now replaced by more powerful chemometric methods. Further, I will stop on our efforts in modeling LIP that we first intended for the improved quality of spectroscopic analysis and later extended to non-spectroscopic fields such as chemical vapor deposition and surface structuring. We developed a version of calibration-free LIBS, in which we iterated model-generated spectra until a close match was achieved between experimental and synthetic spectra to determine concentrations. Next, I will briefly overview our recent developments in plasma modeling that include plasma chemistry. This was important in view of widening application of LIBS as a molecular technique. I will also address several plasma diagnostics, e.g., Radon transform tomography that we developed to get more insight about LIP that was helpful for both analytic spectroscopy and modeling. Finally, I will mention several exotic applications of LIP such as LIP-based lasers and chemical reactors to illustrate a real multifaceted character of laser induced plasma and usefulness of its study for many science fields.

22AES06: Emerging Leaders Session

Chair: David Charlot

(AES-06.1) Towards The Use Of Commercially Available Microfluidic Chips for Zeta Potential Characterization

Jonathan Cottet¹, Josephine O. Oshodi¹, Ariel L. Furst¹, Cullen R. Buie¹; ¹MIT

This work demonstrates the possibility of zeta potential characterization with commercially available microfluidic chips.

Bacteria zeta potential, ζ , is one of the key parameters for the deposition of bacteria on an electrode, also called electrophoretic deposition (EPD) and depends on the strain, suspension composition and pH. Measuring ζ directly with a zetasizer is challenging due to the non-spherical shape of bacteria and the need for a lower conductivity medium which could affect the value of ζ . Electrokinetic measurements are usually performed with microchannels in PDMS [1] or in PMMA [2], both of which require expensive tools for the fabrication of either the mold or the microchannel itself. Reducing the cost and time needed to perform such measurements would enable the broader use of this technique. This challenge could be solved using commercially available microfluidic chips.

Under an electric field \mathbf{E} in a microchannel, the electrokinetic velocity of particles in a fluid is linked to two phenomena: electrophoresis (EP) due to the zeta potential of the particle and electroosmosis (EO) due to the zeta potential of the channel wall. PMMA microchannels (Microfluidic ChipShop) with 200 μl reservoirs together with a voltage sequencer (Labsmith) controlled with LabVIEW were used to characterize the velocity of various

polystyrene beads suspended in 10 mM HEPES (pH 7) by applying between the inlet and outlet platinum electrodes a voltage ramp between two voltage holds. Recorded time-lapse fluorescent image sequences were automatically analyzed using Matlab, FiJi and TrackMate. The slopes of the electrokinetic velocities obtained by Particle Image Velocimetry (PIV) during the voltage ramp were fitted with a linear fit with a high coefficient of determination allowing the extraction of the different particle zeta potentials, which are in good agreement with the control measurements performed with a Zetasizer Ultra (Malvern Panalytical) [3].

The use of commercially available microfluidic chips in electrokinetic characterization could alleviate the need for microfabrication and allow the wider adoption of this characterization method. These measurements are ultimately critical to better understand the role of ζ in EPD of microbes for an array of applications.

[1] S. Antunez-Vela, et al., *Anal Chem*, 2020

[2] Q. Wang et al., *Science Advances*, 2019

[3] J. Cottet, et al. *In preparation*

(AES-06.2) **ESSENCE 2.0: An Improved All-In-One POC Platform**

Yu Husan Cheng¹, Halexandra Alvarenga², Thara Balaji¹, Aditi Sathe³, Zhenglong Li¹, Charmi Chande¹, Sagnik Basuray¹; ¹*New Jersey Institute of Technology*, ²*California Baptist University*, ³*Lehigh University*

Modular POC device as a one-stop clinical instrument to screen infectious diseases, liquid biopsy, etc.

The delayed response in COVID-19 detection during the pandemic has shown the need to develop a universal biosensor platform with adaptive nature for quick response to outbreaks. However, sensors in point-of-care (POC) devices such as ELISA or screen tests suffer from complicated procedures or insufficient sensitivity and selectivity. We have developed the sensor platform, ESSENCE, which uses a Shear-Enhanced, flow-through non-planar 3D Nanoporous Electrode to overcome current electrochemical sensors limitations as a POC sensor, specifically selectivity and sensitivity limitations. The ESSENCE platform consists of a replaceable microfluidic chip and a fully automatic fluidic controlling system. The fluidic platform contains valves, pumps, and pressure sensors, automatically manipulating the sample and buffers' flow and pretreatments. It has been proved from the previous report that this platform has high selectivity and sensitivity for biomolecules like

DNA (fM sensitivity, can distinguish against a mismatched DNA), proteins (breast cancer biomarker p53, fM sensitivity, can distinguish it against another breast cancer biomarker HER2) under the traditional desktop Electrochemical Impedance Spectroscopy(EIS) analyzer in hours. Here we present ESSENCE 2.0, which is completely automated with additively engineered parts and a chip architecture that has been modified to remove any problems related to packing, chip reliability, and signal variability from chip to chip. It shows similar sensitivity and selectivity as ESSENCE 1.0 for DNA and antigens. It shows a promising platform to be a future universal point of use device for every location and used by a regular user.

(AES-06.3) Dielectrophoretic Pressure in Paper (DPiP): A Novel Insulator-based Dielectrophoretic Technique for Low-Cost Trapping and Separation

Md Nazibul Islam¹, Zachary Gagnon¹; ¹*Texas A&M University*

A novel paper-based dielectrophoresis technique that significantly reduces cost and time of device fabrication

Dielectrophoresis and Insulator based dielectrophoresis (iDEP) have received significant attention as tool for selective manipulation of biomolecules for robust and portable micro-total-analysis-systems. However, despite significant academic advances, commercial adoption of DEP-based devices remains low. One reason for such limited adoption is a lack of low-cost manufacturing methods scalable production of fluidic chips. While techniques such as injection molding are capable of scalable production, they often require a six-figure financial investment for both the development and the production phases of commercialization. This constraint severely limits the academic translation, and widespread consumer adoption of DEP-based technology. Therefore, a low-cost and scalable DEP device manufacturing method will have significant impact in democratizing innovation. Here we report a novel iDEP technique called dielectrophoretic pressure in paper (DPiP) that uses paper fibers as insulating structures. We utilize a CO2 laser to cut fluidic channels from sheets of crane glass paper. Using a combination of corona plasma treatment and heat-press, we confine and irreversibly seal these channels and copper-tape electrodes within PDMS membranes. We then flow fluid through paper and apply a pulsed DC electric field across the channel. While passing perpendicular to a paper channel, electric field lines diverge and converge at different zones due to the porous structure of paper. This creates pockets of high field strength within the paper pores that trap particles. Using the abovementioned technique, we can capture polystyrene particles within paper pores with only 10V of DC applied voltage. Using CT-scan images of paper, we have developed a COMSOL model that demonstrates the formation of these high field pockets. The DPiP technique is biocompatible and can be used to capture and concentrate red blood cells, E. coli, and DNA. To the best of our knowledge, this is the first-time paper fibers have been used as insulating structures for dielectrophoretic trapping. This novel technique democratizes DEP-based innovations and allows for robust device manufacturing at the

commercial scale.

(AES-06.4) Using Deep Eutectic Solvents as the Separation Media in Capillary Electrophoresis

Christopher R. Harrison¹, Shreeya Venkatesan¹; ¹*San Diego State University*

Exploration of a new solvent for conducting electrokinetic separations

There has been a growing interest in using deep eutectic solvents (DES) in analytical separations in recent years. DES have been used as both an extraction solvent, allowing for the extraction of target analytes from complex matrices, as well as an additive to separation buffers to improve separation results, and even as stationary phases for chromatographic separations. The incorporation of DES in electrophoretic separations has however been somewhat limited, with DES most commonly being used as extracting solvents, and occasionally included in the separation buffers.

DES can be produced for very low cost, and with ease; DES consist of a hydrogen bond donor and hydrogen bond acceptor pair, mixed in the proper molar ratio. Mixing the donor-acceptor pair, and possibly heating the mixture, is all that is required to form most DES. Varying the donor and/or the acceptor can change the physical and chemical properties of the DES. As a result, the number of DES, and their properties are almost endless.

In this work we will present our exploration of a new DES that is suitable to electrophoretic separations. Most DES are prepared with choline chloride, however this salt renders the DES highly conductive, and electrophoresis very challenging. As such the pure DES is not used in separations, rather it is diluted significantly with an aqueous buffer. Our DES, which replaces choline chloride with the analogous tertiary amine, 2-dimethylaminoethanol, has many advantages. Lacking the chloride, our DES has very low conductivity, allowing the pure DES to be the separation media in an electrophoretic separation. In addition, the acid-base characteristics of the DES allow for the deprotonation of the silica surface, providing a surface charge and EOF. This DES can also be mixed with what to varying percentages to change the solvent, and electrophoretic properties. This work will present our current understanding of this new DES and its applicability to electrophoretic separations.

(AES-06.5) Nonlinear Electrokinetics for Separating Microorganisms

Alaleh Vaghef Koodehi¹, Olivia Ernst¹, Blanca H. Lapizco-Encinas¹; ¹*Rochester Institute of Technology*

Novel strategy for separating highly similar cells with electrokinetics

The separation and identification of microorganisms is an important challenge in many fields, ranging from clinical analysis, food safety, environmental monitoring etc. Probing microorganisms' properties and behavior considering their size, shape, and electrical surface charge can provide valuable information for designing novel separation methods. The field of microscale electrokinetics (EK) offers the possibility of combining several EK phenomena within the same microfluidic device to enable high-resolution separations of microorganisms with similar characteristics. Presented here is the use of insulator-based EK devices (iEK), which use insulators to distort the electric field distribution in a microchannel to combine linear and nonlinear EK phenomena to finetune a desired separation. In this work, we utilized a microfluidic device with asymmetrical insulating posts to separate several microorganisms of interest: *E.coli*, *B. cereus*, *B. subtilis*, *S. enterica* Typhimurium, and *S. cerevisiae*. To design a separation process, we first carefully characterized the EK behavior of these microorganisms under linear and nonlinear EK regimes, and with this information, we built a mathematical model to identify the operating conditions to achieve the separation. The modeling and experimental results have illustrated that accurate characterization and modeling are effective tools for predicting the conditions under which a desired separation will take place. Experimental results showed successful separation of mixtures of cells that were accessed in terms of separation resolution. This work illustrated that iEK systems have the potential for achieving effective separations of microorganisms in a matter of minutes.

22AWD05: NESAS and SAS Lester W. Strock Award Symposium Honoring Igor Gornushkin

Chair: Igor Gornushkin

(AWD-05.1) In-depth Characterization of ICCD Detector for LIBS Measurements

George Chan¹; ¹*Lawrence Berkeley National Laboratory*

Intensified charge coupled device (ICCD) is very likely the most popular detector for laser induced breakdown spectroscopy (LIBS) measurements nowadays, but it is not without shortcomings. Our symposium honoree, Igor Gornushkin, noted the limitations of ICCD long ago and therefore proposed different methods to avoid the use of ICCD, for example, by coupling a non-intensified CCD with a mechanical chopper [1] or an acousto-optical modulator. As noted by Igor and his co-workers, ICCD not only “are more cost-intensive and less robust than nonintensified CCDs” [1], “their susceptibility to blurring the detected images” with “accompanying loss in spectral resolution” [2] is another drawback. Recently, we have performed an in-depth characterization of the noise, gain non-uniformity, and blurring effect of an ICCD detector with an objective to better understand how these factors

affects LIBS measurements, in particular those related to optical isotopic analysis which requires high spectral resolution of comparatively weak signals. In this presentation, results of our characterization will be presented and means to minimize the impacts will be discussed.

1. M. Mueller, I.B. Gornushkin, S. Florek, D. Mory, U. Panne, Approach to detection in laser-induced breakdown spectroscopy, *Anal. Chem.* 79 (2007) 4419-4426.

2. P. Pořízka, B. Klessen, J. Kaiser, I. Gornushkin, U. Panne, J. Riedel, High repetition rate laser-induced breakdown spectroscopy using acousto-optically gated detection, *Rev. Sci. Instrum.* 85 (2014) 073104.

(AWD-05.2) Coupling Laser Ablation and Plasmic Structures for Elemental Analysis
Alessandro De Giacomo¹, Marcella Dell'Aglio²; ¹University of Bari, ²CNR-NANOTEC

In this lecture the plasmon enhanced ablation for elemental analysis is investigated with several experiments in order to discuss the main questions concerning the laser matter interaction upon the effect of plasmonic coupling between the nanoparticle (NP) system and the laser ablation pulse. The correlation between the electromagnetic field enhancement and the signal enhancement during NP enhanced laser induced breakdown spectroscopy (NELIBS) is discussed from experimental and theoretical points of view, as well as the laser matter interaction at the nanoscale, when noble metal NPs deposited on metal samples. The main difference between conventional and NP supported ablation is reported in order to understand the peculiarities of Nanoparticle supported ablation.

(AWD-05.3) Rare-earth Elements Analysis by LIBS

Michael Gaft¹, Lev Nagli¹, Yosef Raichlin¹; ¹Ariel University

The detection of REE by LIBS by atomic and ionic emission in many cases is difficult due to spectral interferences from other members of this group and accompanying elements. Another detection mode is molecular emission. We proved that the molecules of all REE with oxygen appear effective for analytical purposes. The type of the emission spectrum depends on specific element molecular weight and differs for Light and Heavy REE sub-groups. The analytical approach is demonstrated for Gd analysis in ceria (CeO₂) and in permanent B-Fe-Nd magnets. The present sensitivity in Double Pulse mode is approximately 0.5 %.

The presence of molecular emission opens an opportunity for isotopic analysis. We started to research this option. It was found that the width of the molecular emission lines is strongly element dependent. The mostly narrow ones belong to Y, La, Sc, and Lu. All those elements exist in nature or as one stable isotope (Y, Sc) or mostly one stable isotope (La and Lu). Opposite to that, the elements with relatively broad molecular emission contain several stable isotopes. For example, in nature the gadolinium occurs as a mixture of six stable isotopes with close abundances - ¹⁵⁸Gd (24.84 %), ¹⁶⁰Gd (21.86 %), ¹⁵⁶Gd- (20.47 %), ¹⁵⁷Gd

(15.65 %), ^{155}Gd (14.8 %), and ^{154}Gd (2.18 %). The spectroscopy with resolution of 0.02 nm revealed the absence of spectral splitting in monoisotopic elements, while in Gd for individual emission bands at the strongest transition (0,0 transition, System I) became visible peaking at 461.50, 461.56, 461.52, and 461.70 nm.

At the second stage, we applied Molecular Laser-Induced Fluorescence (MLIF) - LIBS combination for REE isotopes analysis. The mostly sensitive are the bands at 633.3 (1,1 transition, System I) and at 481.66 (1,2 transition, System I). Under the pumping at the 446.26 nm region (0,1 transition, System I) two strongest individual lines appear at 463.33 and 446.48 nm (pumping at 446.32 and 446.53 nm), while three found at 481.74 and 481.92 together with 481.99 nm (pumping at 446.38 and 446.65 nm).

The experiments with individual Gd isotopes are under progress.

(AWD-05.4) A Critical Comparison of Laser-Ablation Atomic Absorption Spectroscopy Paradigms

Jonathan A. Merten¹; ¹*Arkansas State University*

(AWD-05.5) LIBS Imaging: Recent Advances and Perspectives

Vincent Motto-Ros¹, Vincent Motto-Ros¹; ¹*Institut Lumière Matière*

The imaging capability of laser-induced breakdown spectroscopy (LIBS) has a high potential in various domains including biology, industry, geology and medicine (figure 1). This approach can be distinguished by its ease in use, multi-elemental capability, detection of light elements, as well as operation at ambient conditions. This is furthermore the only all-optical technique providing space-resolved elemental information with ppm-scale sensitivity and μm -range resolution. These advantages, make LIBS imaging very attractive to be used in research laboratories for routine investigations.

However, advanced technological solutions must be found for this application since elemental imaging requires high sensitivity, sharp spatial resolution, high speed of acquisition as well as the ability to process a huge quantity of data. In this presentation, we will summarize the recent progresses made in the Light and Matter Institute concerning the implementation of the LIBS imaging. In particular, different examples of applications will be shown with the aim of illustrating the specificities and the great potential of LIBS imaging. Different perspectives will be finally discussed.

22AWD08: Coblenz Society Craver Award Symposium Honoring Wei Min

Chair: Wei Min

(AWD-08.1) High Performance Infrared Spectroscopic Imaging for Rapid Biomedical Assessment

Rohit Bhargava¹, Kevin Yeh¹, Seth M. Kenkel¹, Yamuna Phal¹, Anirudh Mittal, Kianoush Falahkheirkhah; ¹*University of Illinois Urbana-Champaign*

The optical microscope has been invaluable for both scientific discovery and diagnostic cancer pathology. Images are typically illustrative of the structural organization of biomedical samples and to visualize biologically-important structures, contrast agents need to be added. Finally, images are often interpreted using human visual cues. Here we describe advances in infrared chemical imaging to provide a feasible technology in which dyes and stains are not needed since the native chemical composition provides image contrast. There are three major technological directions to enable practical technology that need to be balanced - increasing speeds for rapid measurements, increasing capability in data quality (signal to noise ratio, reproducibility and image detail), and providing practical instrumentation. We describe recent advances in laser scanning IR microscopy that achieve these goals for many needs in cancer pathology. Artificial intelligence algorithms are used for parsing the vast data to provide informative views. We provide examples of how modern machine learning techniques are improving information extraction from chemical imaging and providing new capabilities for conventional pathology. We provide examples of how this technology can help understand composition, molecular changes and impact of physio-chemical organization in cancer samples.

(AWD-08.2) Principles of 2D IR Imaging and Applications to Cataract and Amyloid Tissues

Martin Zanni¹; ¹*University Wisconsin-Madison*

This talk will cover hyperspectral 2D IR spectroscopy imaging of human eye lenses and pancreatic tissue of transgenic mice. 2D IR spectroscopy monitors secondary structure through frequencies, like FTIR imaging, but also transition dipole strengths, anharmonicities, polarizations, and cross peaks. The principles behind each of these observables will be explained, and applications to cataract formation and pancreas tissues will be given.

(AWD-08.3) Functional Stimulated Raman Imaging for Complex Subcellular Analysis

Lu Wei¹; ¹*Caltech*

Innovations in optical spectroscopy and microscopy have revolutionized our understanding in live biological systems at the sub-cellular levels. In this talk, I will present our recent advances in developing and applying stimulated Raman scattering (SRS) imaging, a nonlinear vibrational imaging modality that offers rich chemical information, for specific and highly sensitive investigations of complex biological (i.e. cancer- and neuronal-) systems. First, we integrated Raman spectroscopy and imaging with transcriptomics analysis for metabolic phenotyping in cancer systems. Our subcellular Raman-guided strategy revealed potential new druggable targets that are not present in bulk analysis. Our further integrations with lipidomics and transcriptomics suggest possible underlying regulatory pathways. Second, I will discuss our recent efforts to develop a general sample-expansion vibrational imaging strategy for label-free high-resolution (down to 78 nm) chemical imaging in cells and tissues. With further adoption of machine learning training, we successfully obtained label-free, multi-component, and volumetric prediction of nucleus, blood vessels, neuronal cells, and dendrites in complex mouse brain tissues. We envision

this approach will offer an effective and specific way for sub-phenotype profiling especially in large tissue scales.

(AWD-08.4) Super-resolution Multiplexed Metabolic Imaging of Aging and Diseases
Lingyan Shi¹; ¹*UC San Diego*

Understanding the dynamics of metabolism in a multicellular organism is essential to unraveling the mechanistic basis of many biological processes in healthy and diseased conditions. There has been an urgent need of high spatial resolution, non-invasive imaging techniques for imaging metabolism of various biomolecules in cells and tissues. Deuterium oxide probed Stimulated Raman scattering (DO-SRS) can generate chemical specific metabolic imaging with high resolution, deep penetration of depth, multiplex, chemical selectivity, 3D volumetric and quantitative capability. In the present work, we developed a new approach that combines super resolution Adam optimization-based Pointillism Deconvolution (A-PoD) enhanced **SRS** imaging and custom designed clustering methods to visualize multiplex metabolic activities and subcellular distribution of newly synthesized macromolecules in living organisms. Within the broad vibrational spectra, we can image more than 30 different molecules including lipids subtypes-, protein-, and DNA-specific Raman profiles and develop hyperspectral detection methods to obtain multiplex imaging of various biomolecules. This technology platform is non-invasive, universal applicable, and it can be adapted into a broad range of biological studies such as neurodegeneration, aging, homeostasis, tumor progression, etc. We applied this method to study the diet regulated metabolic dynamics in animals during aging processes, the quantitative lipid and protein turnover rate, the intra-cellular metabolic heterogeneity.

(AWD-08.5) Genetics Free Optoacoustic Neuromodulation
Ji-Xin Cheng¹; ¹*Boston University*

We present a fiber optoacoustic emitter for optoacoustic stimulation of neurons with an unprecedented spatial resolution up to 20 microns, enabling selective activation of single neurons or subcellular structures such as axons and dendrites. To enable region specific and high-efficiency cell modulation, we developed a fiber-based optoacoustic emitter (FOE), serving as a miniaturized ultrasound point source, with sub-millimeter confinement. By modifying acoustic damping and light absorption performance, controllable frequencies in the range of 0.083 MHz to 5.500 MHz are achieved and further induce cell membrane sonoporation with frequency dependent efficiency. To achieve neuromodulation at single cell spatial resolution, we further developed a tapered fiber optoacoustic emitter (TFOE) enabling stimulation of single neurons and subcellular structures. The highly confined ultrasound enabled integration of the optoacoustic stimulation with stable patch clamp recording on single neurons for the first time. Cell-type-specific response of excitatory and inhibitory neurons to acoustic stimulation was unveiled. Towards understanding the biomolecular mechanisms of optoacoustic cell modulation, we show that optoacoustic excites primary cortical neurons through specific calcium-selective mechanosensitive ion channels with the assistance of calcium amplifier channel and voltage-gated channels. Pharmacological inhibition of specific ion channels leads to reduced responses, while over-

expressing these channels results in stronger stimulation. These results shed new insights into the mechanism of ultrasound neurostimulation.

22BIM06: Optical Technologies for Disease Screening and Diagnostics

Chair: Fay Nicolson

(BIM-06.1) Point of Care Diagnosis of Preeclampsia

Samuel Mabbott¹, Samuel Mabbott¹; ¹*Texas A&M University*

Childbirth is a unique and beautiful event that, regrettably, is sometimes tainted by the tragic death of the mother or the child. Preeclampsia (PE) is a leading cause of maternal and fetal mortality and morbidity in both well-resourced and under-resourced settings. PE can lead to several chronic diseases in both the mother and her unborn child later in life. Overall, approximately 76,000 mothers and 500,000 babies die every year due to complications of PE. Physicians most commonly diagnose preeclampsia late in gestation when symptoms manifest (around the 20th week) by documenting hypertension or proteinuria in an expectant mother. However, it is too late to either reverse or prevent the progression of the disease. No preventative testing is currently available, and treatment is nominal. Ideally, therapeutic interventions would need to begin before the onset of disease pathology. Thus, early detection of preeclampsia through POC biomarker detection may enable closer monitoring of women at increased risk of developing preeclampsia and help identify candidates for participation in early intervention. To achieve the goal of early detection we have focused on the measurement of preeclampsia associated miRNAs using SERS-enabled point of care platforms. In my talk I will give an overview of preeclampsia, the assays we have designed for targeting preeclampsia associated miRNA, and the platforms developed to enable point-of-care diagnoses.

(BIM-06.2) Breaking Multiplexity Limits of SERS Imaging to Enable Highly Specific Molecular Imaging and Spatial Profiling of Diseased Tissues

Olga Eremina¹, Alexander Czaja¹, Augusta Fernando¹, Arjun Aron¹, Dmitry Eremin¹, Cristina Zavaleta¹; ¹*University of Southern California*

Many facets of medicine require the ability to label abnormal cells to make their presence and properties known – surgery for location and resection of tumors, pathology for diagnosis and subtyping of cancer cases, and pharmaceutical research to characterize responses to experimental treatments. In each discipline, the ability to perform labeling and imaging for a large number of cellular targets would dramatically increase the throughput and quality of biological insight. Clinical pathology today must use the accepted “gold standard” technique, immunohistochemistry (IHC), which marks cells expressing a single biomarker with a brown precipitate. Staining for additional biomarkers must be done on separate subsequent tissue sections using whatever remains of the patient’s sample. Pathology augmented with high content multiplexed imaging could perform more extensive and accurate subtyping of cases on a single tissue section for better treatment selection.

Our answer is to adopt optically unique surface-enhanced Raman spectroscopy (SERS) nanoparticles, as the vehicle to achieve highly multiplexed imaging in a single acquisition,

which is non-destructive, quantitative, and simple to execute. As Raman scattering is significantly amplified by the contrast agents' nanoscale gold core, high signal-to-noise is achieved with short exposures, meaning the scans can be executed quickly and with low, non-destructive laser power. We demonstrate that the SERS nanoparticle concentrations can be accurately quantitated by the intensity of their unique spectral emissions. Our newly expanded library of SERS nanoparticle formulations was thoughtfully curated and designed to minimize each nanoparticle's number of peaks while maximizing the distance between their respective peaks. These are important advancements which maximize their multiplexing performance and allow, for the first time, 26 unique nanoparticle batches to be successfully unmixed while co-localized in a single imaging pixel.

(BIM-06.3) Development of a Compact Dual Raman and Fluorescence Spectrometer for Point-of-Care Diagnostics

Cyril Soliman¹, Jonathan Faircloth², Samuel Mabbott¹, Gerard L. Coté¹, Kristen Maitland¹;
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The development of a compact, dual Raman and fluorescence spectrometer.

The implementation of point-of-care (POC) diagnostics for disease detection have resulted in major strides in bioassay design and testing. As more innovative assay designs trickle down the pipeline, the instrumentation used to measure the signals from these assays have largely remained unchanged. Within the field of Raman spectroscopy, large confocal Raman microscopes continue to serve as the instrument of choice due to their high sensitivity, mapping capabilities and user-friendly interfaces. Alternatively, handheld Raman spectrometers are gaining popularity due to their compact size, sensitivity, and versatility. Within the field of fluorescence spectroscopy, benchtop fluorometers and plate readers are the instruments of choice, but more compact optical systems can be assembled using commercial hardware. Novel approaches in spectrometer design can aid in the translation of these diagnostic platforms from the research laboratory to the POC. One novel approach is the coupling of Raman and fluorescence spectroscopy into a single spectrometer optical bench. The added functionality of fluorescence can enable multiplexing, improve sensitivity, and expand potential application spaces. In collaboration with Wasatch Photonics, we have developed a dual Raman and fluorescence spectrometer based around their innovative grating and spectrometer design. The developed spectrometer has a spectral resolution of 8 cm^{-1} across the Raman region of interest ($200\text{-}2000 \text{ cm}^{-1}$) with a compact form factor and high sensitivity. The multi-modal functionality was validated using gold nanoparticles conjugated with Raman reporter molecules (4-Mercaptobenzoic acid) in solution with fluorescent molecules (fluorescein) across a gradient of molar concentrations ($0\text{-}50 \text{ }\mu\text{M}$). In addition, the potential clinical utility of the spectrometer was demonstrated using a model multi-modal bioassay. The development of the dual Raman and fluorescence spectrometer highlights the versatility of the optical system as a platform technology to enable POC diagnostic applications.

(BIM-06.4) Multimodal Nonlinear Optical Microscopy Unveils Early Therapy-induced Senescence in Human Cancer Cells

Dario Polli¹, Arianna Bresci¹, Francesco Manetti¹, Silvia Ghislanzoni², Federico Vernuccio¹, Chiara Ceconello¹, Benedetta Talone¹, Alejandro De La Cadena¹, Subir Das¹, Renzo Vanna³, Italia Bongarzone², Giulio Cerullo¹; ¹*Politecnico di Milano*, ²*IRCCS Istituto Nazionale dei Tumori Foundation*, ³*CNR-Institute for Photonics and Nanotechnologies (IFN-CNR)*

Multimodal nonlinear optical microscopy reveals therapy-induced senescence in tumors, monitoring the risk of cancer relapse.

Recent advancements in cancer research revealed that radio- and chemo-therapy can induce a condition of tumor cell senescence rather than cell death, constituting one of the major causes of cancer relapse. The development of non-invasive, accurate and clinically translatable tools to monitor the presence of therapy-induced senescent (TIS) cells is thus crucial to evaluate the effectiveness of anticancer treatments and prevent tumor recurrence.

Here, combining different coherent Raman and multi-photon processes, we demonstrate label-free multimodal nonlinear optical (NLO) microscopy as an effective technique to unveil early-stage TIS traits and monitor them, quantitatively and non-invasively. We home-built a multimodal microscope featuring seven different NLO modalities, along with linear transmission light: forward-detected Stimulated Raman Scattering (SRS), forward and epi-detected Coherent Anti-Stokes Raman Scattering (CARS and E-CARS), Two-Photon Excited Fluorescence (TPEF and E-TPEF) and Second-Harmonic Generation (SHG and E-SHG). The laser source delivers 780 nm pump pulses and 950-1050 nm tunable Stokes pulses with 1 picosecond duration, matching the CH-stretching region of the Raman vibrational spectrum (2800-3100 cm^{-1}).

The combination of these co-registered microscopy techniques on both deferoxamine (DFO)-induced senescent cells and untreated controls unveiled label-free quantitative hallmarks of early-stage TIS, corroborated by comparing different modalities and monitored over 7 days of therapy. TPEF from NADH/FAD combined with E-CARS at 2850 cm^{-1} from cardiolipin showed a disaggregation of TIS mitochondria (p-value < 0,001 at 24 hours of therapy). CARS and SRS at the 2850 cm^{-1} Raman mode of lipids revealed an accumulation of lipid droplets in TIS cells (p-value < 0,001 at 72 hours of therapy). TIS nuclei showed irregular spreading, as visualized via subtraction of 2930 cm^{-1} and 2850 cm^{-1} SRS signals of proteins and lipids, confirmed by CARS imaging at the 2970 cm^{-1} CH vibration of deoxyribose.

The early-stage TIS markers here presented agree with qualitative senescence markers previously reported via invasive and destructive techniques at later stages of TIS. We consider that our findings will be extremely valuable resources for the future of clinical anticancer protocols and non-invasive biomedical diagnostics, allowing one to easily, quickly and non-invasively reveal early-stage TIS and the related risk of recurrence in treated human tumors.

(BIM-06.5) Enhanced Tri-modal Optical-Photothermal Infrared (O-PTIR) Spectroscopy – Advances in Spatial Resolution, Sensitivity & Tri-modality (IR, Raman & Fluorescence)

Mustafa Kansiz¹; ¹*Photothermal Spectroscopy Corp*

Novel combination of IR, Raman and Fluorescence microscopy presented to provide enhanced IR spatial resolution

Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a cutting edge vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopic tools, providing submicron simultaneous IR+Raman microscopy, in non-contact mode with high sensitivity. The ability to collect, for the first-time submicron IR spectroscopic data in an optical microscope has enabled new research outcomes across a range of application fields, such as life sciences (cells, tissues, bacteria), polymers, cultural heritage and microplastics.

A new modality, “counter-propagating” has been engineered to provide for enhanced IR (and Raman) spatial resolution and sensitivity, through decoupling the need for a reflective objective. The IR pump beam can now be directed to the sample via the underside, thus allowing the collection objective for the visible probe (and Raman excitation beam) to be a high-NA refractive objective. This improves spatial resolution to ~300nm for both IR and Raman, whilst improving sensitivity, image quality and facilitating immersion objective studies.

To further integrate vibrational spectroscopic tools into life science workflows, we coupled widefield epifluorescence to facilitate a novel concept – fluorescence guided (or fluorescence co-located) O-PTIR microspectroscopy. Rather than, or in addition to the visible image, the fluorescence image can now be used to guide the user to the region of interest, thus combining the well-established specificity of fluorescence imaging with the broad macromolecular profiling capabilities of IR spectroscopy

Several life sciences examples from bacteria, cells and tissues will be provided to demonstrate these new capabilities and how they can enable new experiments and research findings.

22FORENS04: Pharmaceutical Forensics

Chair: Adam Lanzarotta
Co-Chair: Alexis Weber

(FORENS-04.1) Parallel Column Gas Chromatography combined with Mass Spectrometry for Comprehensive Forensic Analysis of Benzodiazepines

Matthew R. Wood¹, Matthew R. Wood¹; ¹*Ocean County Sheriff's Office, New Jersey*

In law enforcement drug seizures, there has been a surge in the prevalence of counterfeit benzodiazepines (BZDs), where designer benzodiazepines have been substituted in order to mimic authentic pharmaceutical preparations. The similarity in the parent structure of BZDs makes them difficult to separate on a general controlled substance gas chromatography-mass spectrometry (GC-MS) screening method. The purpose of this research was to develop and validate a comprehensive GC-MS method that utilizes parallel gas chromatography to separate and identify a panel of 45 different BZDs and designer BZD analogues. Gas chromatography parameters including inlet temperature, temperature program, and split ratio were altered to maximize resolution and minimize method run time. The optimized method has a run time of 20.00 minutes and provides satisfactory resolution for 63% of consecutively eluting compounds on the Restek Rxi-5ms column. This method is designed to utilize confirmation columns to separate and identify compounds subject to co-elution on the Rxi-5ms column. The Rxi-200ms and Rxi-35ms columns both provide satisfactory resolution for 76% of the co-eluting compounds when paired with the Rxi-5ms column, or 90% of pairs overall. All three columns used together provide satisfactory resolution for 88% of co-eluting compounds, or 95% of pairs overall.

(FORENS-04.2) Field Deployable Analytical Toolkit for Rapid Analysis of FDA Regulated Products at International Ports of Entry

Sara E. Kern¹, Adam Lanzarotta¹, JaCinta Batson¹, Michael Thatcher¹, Martin K. Kimani², Lisa Lorenz¹, Brian Boyd¹, Melissa Collins¹, Anvi Patel¹, Julio Arrecis¹, Kelsey Griffin¹, Fernando Gonzalez¹, Gregory Howe¹, Morgan Hudson-Davis, Mark Loh¹, Flavia Morales¹, Allison Taylor¹, Anthony Wetherby¹, Muhammed Altaf¹, David Laguerre¹, Donna LaGarde¹, Valerie Toomey¹; ¹*US Food and Drug Administration*, ²*U.S. Food & Drug Administration*

In fiscal year 2020, over 729 million units of international mail were processed by the United States Postal Service (USPS), and many contained potentially dangerous unknown, unapproved and misrepresented drug products under the purview of the US Food and Drug Administration (FDA). To increase the number of products inspected and protect consumers, the FDA's Forensic Chemistry Center (FCC) launched a satellite laboratory program at the Chicago International Mail Facility (IMF). Two analysts permanently staff this laboratory and analyze samples for the presence of active pharmaceutical ingredients (APIs) using an analytical toolkit that was extensively evaluated for ruggedness, ease of use, and speed during a pilot study. This toolkit consists of handheld Raman and portable FT-IR spectrometers, and a portable ambient ionization source coupled to a mass spectrometer that has detected over 250 unique APIs in drug products seized during the pilot and production program. This program was originally implemented to target opioids, particularly fentanyl and fentanyl analogs, but has evolved to include any type of FDA regulated product with an

emphasis on complete unknown samples without labeling, which can be challenging even in a traditional brick-and-mortar lab with an arsenal of well-established techniques. Over the course of 200 working days, 479 samples were completed and in 73.3% of the samples, at least one API was detected by at least one of the devices in the toolkit; these samples were submitted for possible regulatory action. Of the 401 APIs detected in these samples, several were either unapproved, controlled substances, and/or fall under the scope of section 801(u) of the Food, Drug and Cosmetic Act, which are drug products that have been determined to pose a significant public health concern. A total of 62 samples for which the toolkit yielded inconclusive results were sent to a traditional FDA lab for confirmatory analysis.

Here we delve into the relative merits and limitations of each device, describe the ongoing optimization of the workflow, examine the results for the items analyzed in the satellite laboratory to date, and the items that underwent confirmatory analysis, and discuss plans for future satellite laboratories located at other IMFs.

(FORENS-04.3) The Advantages of Integrating Portable Spectrometers for Counterfeit Detection and Analysis Casework

Pauline E. Leary¹, Richard Crocombe², Pauline E. Leary¹, Brooke W. Kammrath³; ¹*NOBLE*, ²*Crocombe Spectroscopic Consulting, LLC*, ³*University of New Haven*

The infiltration of counterfeit drugs in the pharmaceutical supply chain is dangerous because these goods (1) may not provide therapy to the patient, (2) may provide too much or too little of the intended active ingredient, or (3) may even cause death or harm to the patient when dangerous chemicals are used as replacements for the intended therapeutic pharmaceutical. The increase in the availability of portable spectrometers over the past two decades has enabled pharmaceutical and forensic scientists analyzing these drugs to obtain meaningful data in real time at the sample scene. This capability can not only be used to quickly detect counterfeits, but also can be used to support investigations and minimize laboratory backlogs by informing evidence selection at the scene.

In most instances, the availability of these spectrometers began due to a need in other market spaces and were then extended to the analysis of counterfeit drugs. For instance, mid-infrared and Raman spectrometers were initially deployed to first responders at the scenes of hazardous materials. Gas chromatograph-mass spectrometers were initially used to analyze hazardous environmental pollutants in the field. These and other technologies have since been applied successfully to the analysis of counterfeit drugs and, due to the ability to analyze samples at the scene, can provide data and results that can greatly improve the quality of the processing of scenes where these goods have been encountered. Cases where portable spectrometers have been successfully deployed to detect and identify counterfeits in the field will be used to describe and highlight the value of portable spectrometers for the counterfeit detection and analysis casework.

(FORENS-04.4) Protecting Patients Using Forensics and Innovative Technologies

Ravi Kalyanaraman¹; ¹*Bristol Myers Squibb*

Pharmaceutical Forensics ensures that patients receive high quality and safe products.

Forensics describes the scientific methods used in an investigation. Pharmaceutical forensics is looking for evidence and using your scientific knowledge and know-how to find proof that will help solve issues in manufacturing, patient safety, and crimes. Forensics and Innovative Technologies (FIT) group was created in 2021 within Global Quality Analytical Science and Technologies (GQAS&T) to support commercial operations for investigation support in manufacturing, patient complaints, and to screen suspect and counterfeit products. Several state-of-the-art analytical tools and techniques are used to support pharmaceutical forensics. These include but not limited to Energy Dispersive x-ray Spectroscopy (EDS), Scanning Electron Microscopy (SEM), confocal Raman, portable and benchtop Raman, Infrared (IR), Near Infrared (NIR) micro-spectroscopy, and Quantum Cascade Laser (QCL) IR spectroscopy. Visible NIR (VNIR) Hyperspectral Imaging (HSI) technique has been recently added to this toolbox to rapidly screen counterfeit drugs and also to identify and characterize particulate and foreign matter in drug products. The talk will feature case studies along with the support provided by FIT for commercial products which includes pharma, biologics and cell therapy products.

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22LIBS08: Medical Applications

Chair: Pavel Porizka

Co-Chair: Jozef Kaiser

(LIBS-08.1) Determination of Elemental Distributions within Functionalized Polystyrene Beads

Andreas Limbeck¹, Birgit Achleitner, Aida Fazlic, Davide Ret, Simone Knaus; ¹*TU Wien, Institute of Chemical Technologies and Analytics*

Glycosylation is an important and highly regulated mechanism of secondary protein processing within cells. It plays a critical role in determining protein structure, function and stability. 70% of all human proteins bear carbohydrate chains (glycans). These glycans play an essential role in many important biological processes and have been associated with a number of diseases. Therefore, analysis of structurally diverse glycans from biological samples represents an essential field for the investigation of their structure-function correlations.

In general, almost all glycan samples must undergo several tedious purification steps prior to analysis. A facile and versatile method for the quantitative isolation of glycans from complex samples is using sulfonyl hydrazine-functionalized polystyrene (SHPS) beads. These beads are synthesized via various steps, starting from sulfonated polystyrene (PS) ion exchanger beads to obtain a hydrazide activated surface. Hydrazide activated PS beads can be produced in large amount, high yield and allow an easy and fast purification of free reducing end glycans from complex samples mixture for spectrometry analysis.

During the manufacturing process, the product and all intermediate steps are measured by IR spectroscopy. This approach allows appropriate characterisation of bulk samples, but the applicability for the investigation of single polystyrene beads is limited, especially a differentiation between surface and core of the beads is not possible.

In this contribution LIBS has been applied for an improved characterisation of the individual reaction products. In a first step of the synthesis procedure the counter ion (Potassium) of the ion exchange resin used as starting material is released, in the subsequently performed chloro-sulfonation the element Chlorine is introduced, which is afterwards substituted with a hydrazine group resulting in the final product. Careful optimisation of the applied LIBS parameters allowed spatially resolved measurements of the investigated elements, a prerequisite for the further improvement of the synthesis procedure.

(LIBS-08.2) Towards Cohort Study of Cutaneous Cancers Using Laser-Induced Breakdown Spectroscopy

Hana Kopřivová¹, Kateřina Kiss², Jakub Buday¹, Lucie Vrlíková³, Milan Kaška², Marcela Buchtová³, Jozef Kaiser¹, Pavel Porizka⁴; ¹Central European Institute of Technology, Brno University of Technology, ²Faculty of Medicine in Hradec Kralove, Charles University, ³Academy of Science of the Czech Republic, ⁴CEITEC Brno University of Technology

The skin is the largest organ of the human body that serves as a protection from external influences. These adverse environmental influences may be the reason for skin to develop from various diseases, such as cancer. A tumor can generally be described as an unhealthy tissue in which body cells divide and grow uncontrollably and spread to other parts of the body. According to the researchers, cancer-infected tissues change the shape of the cells and their chemical composition. These changes in elemental composition can be observed using LIBS, where imaging of biotic elements (Ca, Mg, P, C, and Na) provides information about the distribution of soft tissue elements and, consequently, the location of cancerous tissue.

Laser-induced breakdown spectroscopy (LIBS) is a quasi-destructive analytical method with extensive elemental analysis capabilities. LIBS uses a high-frequency pulsed laser (typically an Nd:YAG). The laser pulse is focused into a tight spot inducing high-intensity irradiation of the material and its ablation; consequently, a luminous micro-plasma is created. The laser pulse parameters play a crucial role in the laser-matter interaction and contribute to the complex phenomenon of plasma formation and its thermodynamic properties. In contrast to commonly used methods that focus on mapping the sample's surface, LIBS allows high-speed imaging experiments with frequencies in the kHz range and spatial resolution down to several micrometers. In recent years, this technique has become increasingly popular for the challenging analysis of soft cancer tissues.

This work builds on previous work from our team. It deals with the most aggressive type of skin cancer, melanoma, associated with a very high mortality rate. Nearly 20 human skin melanoma samples were studied using the LIBS method. In these samples, common features that could lead to the possibility of diagnosing melanomas by LIBS were thoroughly investigated.

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(LIBS-08.3) Looking for Laser-Induced Breakdown Spectroscopy Signatures of Diseases in Biomedical Fluids: Progress and Challenges

Noureddine Melikechi¹, Noureddine Melikechi¹, Joshua E. Landis, Khaoula Ouarak, Helmar Adler, Souheyr Meziane, Kim Berlo, Florentine Zwillich, Erin Gibbons, Farhad Pourkamali-Anaraki, Danielle Bonito, Gregory E. Chiklis, Weiming Xia; ¹*University of Massachusetts Lowell*

Looking for Laser-Induced Breakdown Spectroscopy Signatures of Diseases in Biomedical Fluids: Progress and Challenges

Noureddine Melikechi*, Joshua E. Landis, Khaoula Ouarak, Helmar Adler, Souheyr Meziane, Kim Berlo, Florentine C. Zwillich, Erin F. Gibbons, Farhad Pourkamali-Anaraki, Danielle Bonito, Gregory R. Chiklis, Weiming Xia

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Abstract: Laser-Induced Breakdown Spectroscopy (LIBS) has been applied successfully in a variety of fields and environments. This is in large part due to its ability to provide rapidly and with no or minimal preparation qualitative and quantitative multi-elemental compositions of samples. However, its application to the study of medical samples can be described as nascent. Recently, we have reported on the use of LIBS for the classification of biomedical fluids samples of three distinct cases: patients suffering from cancer and healthy controls, patients known to have Alzheimer's disease and healthy controls, and patients known to have been infected with the SARS-CoV-2 virus and those who have not had SARS-CoV-2. Currently available procedures for the early diagnosis of these diseases are inadequate as they rely mostly on invasive approaches. We will present progress made in the application of LIBS and machine learning to classify and differentiate biomedical fluid samples acquired from healthy controls and diseased ones. We will show that in the case of the SARS-CoV-2 study, the fusion of the LIBS data with Inductively Coupled Plasma-Mass Spectrometry data yields key information that can be used for the classification. Although in its infancy, this work could inspire the development of rapid, unbiased, and minimally invasive techniques for screening, early diagnosis, and improve the management of cancers, neurological and possibly infectious diseases.

(LIBS-08.4) In Situ Multi-Elemental Imaging with LIBS for Periprosthetic Tissue Characterization

Benoit Busser¹, Vincent Gardette², Lucie Sancey³, Pat Campbell⁴, Vincent Motto-Ros²; ¹*Institute for Advanced Biosciences*, ²*Institut Lumière Matière*, ³*Institute for Advanced Biosciences*, ⁴*University of South California*

In human periprosthetic tissues, LIBS multielemental imaging revealed the chemical composition of the defective implant.

INTRODUCTION:

Histopathology analysis of periprosthetic tissues is performed after revision surgery, in an attempt to investigate the origin of the implant failure. Identification of visible wear particles in the tissue relies on size/color/form criteria, and thus, on the pathologist's experience. To investigate the elemental composition of such particles, the need for a complementary technique is mandatory. We developed an all-optical multi-elemental imaging technology based on laser induced breakdown spectroscopy (LIBS) to study metal wear debris, particles and corrosion products in periprosthetic tissues, from a range of human hip arthroplasty revision cases.

METHODS:

Tissues were selected from the institutional review board approved specimen archive of UCLA. They were chosen to represent a spectrum of wear debris types, based on implant retrieval findings and background knowledge of the case. These included tissues with corrosion products or with high Titanium (Ti) or Cobalt Chromium alloy (CoCr) component wear with easily visible particulates to low wearing bearings without discoloration and rare visible particles. LIBS analysis was performed in a pixel-by-pixel manner @100Hz, with a 50 μ m resolution and on the entire specimens' surface (several cm²). We created semiquantitative elemental images for the main foreign elements of interest, i.e. Ti, Cr, and Co.

RESULTS:

Quantification of visible particles with a microscope is a tedious task for the pathologists. By contrast, the LIBS elemental analysis easily showed interesting distribution profiles for Ti, Cr, and Co originating from the implants, in all periprosthetic tissues.

DISCUSSION:

LIBS allowed to detect the chemical composition of metal particles and corrosion products in periprosthetic tissues. In the series of cases described above, areas that appeared devoid of particles on the H&E slides were often demonstrated to contain elements of interest. LIBS can detect submicron, nanometer and soluble ion-protein complex forms of biomaterials as well as endogenous particles that are undetectable at the light microscope level. The method is performed in ambient conditions and offers a fast, cheap and minimally-destructive

alternative to other spectroscopic methods. With further refinement, this may become a valuable tool in the histopathological examination of orthopaedic device related tissues.

(LIBS-08.5) Use of Spectroscopic and Tomographic Techniques for the Detection of Microplastics in Human Tonsils

Viktória Parobková¹, Michaela Kavkova¹, Daniel Holub¹, Gabriela Kalčíková², Pavel Porizka³, Jozef Kaiser¹, Tomáš Zikmund¹, Milan Urík⁴; ¹*Central European Institute of Technology, Brno University of Technology*, ²*University of Ljubljana Faculty of Chemistry and Chemical Technology*, ³*CEITEC Brno University of Technology*, ⁴*Masaryk University - Faculty of Medicine, Department of Paediatric Otorhinolaryngology*

Microplastics (MPs) can also be found in daily-life products and are a highly diverse category of contaminants existing in various sizes and forms. The MP pollution represents an emerging global change threat to the terrestrial ecosystem, including human health. MPs may induce physical, chemical, and microbiological toxicity with a potential negative effect on surrounding tissue. Particles may enter the human body through inhalation and ingestion; small particles (< 2.5 µm) can even penetrate cell. Therefore, it becomes essential to develop analytical methodology that will be capable to detect these MPs within selected human organs. However, the localization and characterization of MPs remain challenging as important information about the local distribution is inevitably lost while using specific methods. In this work we deliver optimized protocol for detection of various MPs by using complementary spectroscopic and tomographic methods. First, we have collected human tonsils and artificially administered MPs into the tissue. Then, X-Ray Computed Tomography (XCT) was used to localize the particles in the bulk of the tissue. Samples were then diluted in potassic acid and filtered. Finally, MPs were located on the filter paper and classified by Raman spectroscopy. Moreover, we demonstrate the feasibility of Laser-Induced Breakdown Spectroscopy (LIBS) for detection and characterization of MPs as an alternative complementary tool in the analytical pipeline. The confidence in the proposed methodological approach and its feasibility for monitoring MPs in biotic samples is based on a current feasibility study that has already been conducted by using clinical samples.

22LIBS10: Instrumentation

Chair: Mohamad Sabsabi

(LIBS-10.1) Underwater-LIBS: From Laboratory to Deep Sea Towards the Applications

Ronger Zheng¹, Yuan Lu¹, Jinjia Guo¹, Ye Tian¹, Wangquan Ye¹, Ying Li¹; ¹*Ocean University of China*

The ocean is a wide field to be explored with highly demanding novel technologies, and spectroscopic techniques are promising in this field. Laser-induced breakdown spectroscopy (LIBS) has been considered as a practical method for ocean applications due to its great capabilities of in-situ, real-time, on-site, multi-component measurement, *etc.* In recent years, we have developed several LIBS and Raman systems and deployed into the deep sea

with the aid of remotely operated vehicle (ROV) and human occupied vehicle (HOV). A prototype LIBS system was developed in our laboratory in 2014. Based on that, an upgraded system LIBSea was deployed on ROV for hydrothermal vent investigation in 2015, and a full-depth detection was carried out from sea surface to seafloor (1800 m). In 2018, an optimized LIBS instrument was made for the cold seep detection, and the carbonatite was firstly analyzed in the deep-sea. Besides that, a LIBS probe was manufactured in 2020 to realize solids analysis with a capability of precise focusing, and in 2021 that system was successfully deployed on HOV to achieve a quantitative analysis of metal alloys. To improve the capability of LIBS in its sea applications, many efforts have also been made in the laboratory, including single-pulse and double-pulse LIBS with special attentions on the impacts of oceanic high-pressure conditions. The relevant typical results will be given in this talk, and the opportunity and challenge for LIBS being an in-situ technique in deep-sea applications will also be discussed briefly.

(LIBS-10.2) Tailored LIBS Systems For Industrial Applications

Reinhard Noll¹, Joachim Makowe¹, Volker Mörkens¹, Markus Dargel¹; ¹*Laser Analytical Systems & Automation GmbH*

Overview of novel LIBS systems for in-line analyzing tasks in various industrial applications.

The development of tailored LIBS systems for industrial applications offers the highest potential for inline analytical measurements in terms of efficient integration in the process environment, operability, automation and data evaluation. LSA as a spin-off company of the Fraunhofer Institute for Laser Technology (ILT) in Aachen, Germany, has gained more than 15 years experience in the development of robust and powerful LIBS systems for industrial applications. LSA developed its own spectrometer platform as a key element of these systems, enabling broadband (vacuum UV to VIS), high resolving (20–80 pm) and high speed detection (up to kHz-regime) of e.g. line-rich LIBS spectra for multi-element analysis. An overview will be given comprising LIBS systems already in routine industrial use and systems currently under development in the frame of European research projects:

- a) Scanning high-speed LIBS for spatially resolved determination of microscopic element distributions – including light elements – in metallic samples, such as inclusions and segregations, with scan areas up to 400 mm x 260 mm, step sizes of 20 µm and 1 kHz single event detection.
- b) In-line monitoring of salt streams with an overhead LIBS unit to determine main salt components (Ca, K, Na, Mg); plasma excitation by DPSSL with 100 Hz.
- c) Drill-core analyzer for soil samples equipped with a modular set of spectrometers covering 170- 880 nm, scan length up to 1 m.

- d) In-line characterization of scrap charges of steel in lorries as well as aluminum and lead scraps on conveyors (EU project: Retrofitting equipment for efficient use of variable feedstock in metal making processes - REVaMP).
- e) Mobile LIBS analyzing device for raw materials to determine in-line the value of ores; capable of being integrated in mining machines (EU project: Insitu ore grading system using LIBS in harsh environments - INSite).
- f) In-line characterization of refractory materials, batching and sorting; results of feasibility test with a pilot plant equipped with picking and discharging by delta robots (EU project: Refractory sorting using revolutionising classification equipment – ReSoURCE).

(LIBS-10.3) 2D LIBS Elemental Mapping Analysis of Steel and Li-ion Battery Electrodes using Pico-Second Laser Irradiation

Yoshihiro Deguchi¹; ¹*Tokushima University*

The analysis of elemental composition distribution is indispensable to ensure the quality of various materials production. In this regard, 2D mapping techniques using LIBS are widely used in various fields. In many LIBS studies, lasers with ns pulse widths are used, and the spatial resolution of 2D LIBS mapping is several to several tens of micrometers. In LIBS using ns laser beams, it is difficult to improve the spatial resolution due to thermal effects during the laser ablation process of the target material. In this study, a spatial resolution of 1 μm was achieved by using a laser with a pulse width of 9 ps. This LIBS mapping system consists of a picosecond laser, lens, spectrometer, ICMOS camera, and XYZ stage. The LIBS system was applied to steel and Li-ion battery electrodes for 2D elemental distribution analysis. The steel sample has a structure with Zn coating around the steel at the μm level, and this LIBS mapping system was able to detect the Zn distribution around the steel at the μm level. This result was in good agreement with the SEM-EDS measurement. LIBS has the advantage of elemental composition mapping without the need for high vacuum conditions and has many advantages when applied to industrial processes. In the future, the LIBS analysis speed is improved to 1 kHz and the LIBS system is utilized for elemental composition mapping in industrial processes.

(LIBS-10.4) An Overview of LIBS Instrumentation with Focus on Mining Applications

Paul Bouchard¹, André Beauchesne¹, Francis Boismenu¹, Antoine Hamel¹, Christian Padioleau, Kim Renaud, Tony Vaillancourt¹, Josette El Haddad¹, Daniel Gagnon¹, Aissa Harhira¹, Elton Soares de Lima Filho¹, Francis Vanier, Mohamad Sabsabi¹; ¹*National Research Council Canada*

The Laser-Induced Breakdown Spectroscopy (LIBS) technique involves several fields of science, such as laser–matter interaction, plasma physics, atomic physics, plasma chemistry, spectroscopy, electro-optics, and signal processing. The LIBS plasma is transient, unlike an inductively coupled plasma, arc plasma or glow discharge plasma, which are all stationary. This characteristic dictates some restrictions on the ability to transfer tools used with other

emission spectroscopy techniques to LIBS. Therefore, the development of LIBS over the years has been closely tied to the development of enabling tools and ongoing improvements in their performance.

As LIBS enabling tools (such as pulsed lasers, detectors and spectrometers) have rapidly evolved in the recent years, LIBS has found its way across a variety of applications and disciplines in geology, planetary science, defense, food, environment, industry, mining, biology, etc. The miniaturization of LIBS equipment has opened new opportunities to perform real time measurements and respond to emerging needs under conditions in which conventional techniques cannot be applied. The advent of new compact components makes the technology more accessible in terms of robustness, low cost and analytical performance to deliver its benefits for real time analysis. There are more than 14000 papers related to the LIBS field dealing with many technological aspects for fundamental purposes, applied and industrial research.

During the last three decades, extensive research has been carried out to improve LIBS performance. Meanwhile, dynamic technological development in the field of solid state lasers, electro-optical detectors, and signal processing was successfully harnessed for LIBS. The analytical performance of LIBS for a multi-elemental analysis now achieves a level that is equal, or even in some cases better, than that of classical methods. LIBS is currently considered one of the most active research areas in the field of analytical spectroscopy.

In this talk, we will present an overview of LIBS instrumentation with focus on its use in mining applications. We will discuss the LIBS instrumentation in terms of robustness, analytical performance and comparison to conventional techniques.

22PAT03: Advances in On-Line Process Analysis

Chair: Xiaoyun (Shawn) Chen

(PAT-03.1) Real-Time In-Line Moisture Determination of High Rubber Graft (HRG) Acrylonitrile Butadiene Styrene (ABS) Resin in a Fluid Bed Dryer

Yusuf Sulub¹, Dejin Li; ¹*SABIC*

This paper presents the development of a robust real-time, in-line determination of High Rubber Graft (HRG) Acrylonitrile Butadiene Styrene (ABS) resin moisture levels in a fluid bed dryer (FBD). Combining chemometrics and near infrared (NIR) spectroscopy, moisture levels of HRG powders were extracted from the acquired spectra. A Metrohm NIR analyzer with a probe inserted in the FBD was used in this study. Calibration samples were prepared in the laboratory using a benchtop FBD to generate powders with varied moisture levels ranging from 0.39 to 44.00%. Calibration spectral measurements were acquired in an offline static mode by placing powders on the probe tip. In addition to the offline data, a subset of in-line FBD NIR spectral data was augmented to the calibration set in order to incorporate dynamic process information. To minimize the non-moisture related spectral interferences, the acquired NIR spectra were preprocessed using multiplicative scatter correction (MSC) and Savitsky-Golay second derivative. A multivariate Partial Least Squares (PLS) approach was used to generate a robust model used to quantitate and predict the moisture values in

real-time during the drying process with a root mean square error of prediction (RMSEP) of 1.23%.

(PAT-03.2) Globally Monitoring 9,000+ Molecular Groups in Whole Crude Using Spectroscopy

Bryan Bowie¹, Bryan Bowie¹, Chad Chrostowski, Payman Pirzadeh; ¹*ExxonMobil*

Fast determination of the composition of whole crude and refinery streams is a valuable tool as it can help avoid disruptions and assess its value. However, the composition is often comprised of tens of thousands of different molecules making it far too complex to thoroughly evaluate in a period of minutes. Structure oriented lumping models (SOL) has been used in the past and allows for the simplification of a complex composition. Each whole crude or refinery stream can be described using ten thousand or so of these lumps reducing the complexity.

The FT(N)IR spectrum of an uncharacterized refinery stream can be described as a linear combination of library of FT(N)IR spectra of known refinery streams whose compositions have been characterized. This creates a blend of known spectra which represents the unknown. Likewise, their respective compositions can also be blended together. Furthermore, a least-squares approach permits easy inclusion of new samples with minimal need for optimization and rehashing of more complex models such as PLS. Least-squares models can also be further augmented with input from other online systems such as density and sulfur to improve accuracy. An FT(N)IR spectrum can be collected in a matter of minutes allowing for a rapid determination of composition. Replicating this at several refineries across the globe, allows for larger scale optimization.

(PAT-03.3) Influence of Powder Stream Density on Near-Infrared Measurements upon Scale-up of a Simulated Continuous Process

Natasha L. Velez-Silva¹, Carl A. Anderson¹, James K. Drennen, III¹; ¹*Duquesne University*

In-line characterization of powder density variation is achieved to facilitate NIR model robustness against scale.

Near-infrared spectroscopy (NIRS) has been widely recognized as a powerful process analytical tool for monitoring chemical composition of powder streams in continuous pharmaceutical processes. Quantitative NIR methods are, however, sensitive to changes in the physical properties of powders, which can decrease model prediction performance. The density variation introduced when NIR measurements are performed on dynamic powder streams at different flow rates is a potential source of a lack of model robustness. Since different powder flow rates are often necessary to match the production requirements (e.g. during scale-up) of a continuous process, the development of efficient strategies to

characterize, understand, and mitigate the impact of powder density on NIR measurements is highly desirable. Efforts in this direction are usually limited by the lack of in-line methods for characterizing powder stream density in real-time. This study focused on assessing the effect of powder stream physical variation on NIR measurements by enabling the in-line characterization of powder density. A powder blend was run through a loss-in-weight feeder and quartz tube assembly, delivering continuous powder streams at various levels of flow rate (15-30 kg/h) and tube angle (37-43° below horizontal) in a 3² full-factorial design. The powder stream was monitored simultaneously by in-line diffuse reflectance NIRS, live imaging and dynamic mass measurements. NIR spectra were collected through the quartz tube using a diode-array NIR spectrometer with a bifurcated fiber optics probe. Live video frames were collected using a CCD camera and LED light panel system to determine powder stream volume via image analysis. The dynamic powder mass measurements, along with the volume, were used to estimate powder stream density. Two-way ANOVA with interaction was implemented to assess the effect of process parameters (flow rate and tube angle) on powder density. Partial least squares regression was used to characterize the influence of both process parameters and powder stream density on NIR spectra. Statistical analysis and multivariate modeling confirmed powder density as a significant source of spectral variability due to flow rate. Besides providing a broader process understanding, results elucidated potential mitigation strategies to facilitate effective continuous process scale-up while ensuring NIR model robustness against density.

(PAT-03.4) How the chemical recovery process in the pulp and paper industry may profit from in-line Raman spectroscopy

Karin Wieland¹, Anna Katharina Schwaiger², Barbara Weiß¹, Bernhard Lendl³, Martin Kraft¹; ¹Competence Center CHASE GmbH, ²Sappi Europe | Gratkorn Mill, ³TU Wien

Raman spectroscopy is a valuable tool for in-line measurements in the pulp and paper industry.

Recovery of chemicals used in the solubilization of cellulose from wood is a key process technology in the pulp and paper industry for both ecological and economic reasons. The main chemical ingredient of the “cooking liquor” used in this solubilization is Mg(HSO₃)₂, plus high concentrations of SO₂*H₂O and other cations such as Ca²⁺ and Na⁺. To recover the SO₂, the spent cooking liquor, which contains high amounts of organics, is incinerated and the SO₂ is recovered from the hot flue gas in a cascade of Venturi scrubbers. Exposing the flue gas to a Mg(OH)₂ slurry, the magnesium bisulphite cooking liquor is formed essentially following the 2-step reaction $Mg(OH)_2 + 2 SO_2 \rightarrow MgSO_3 + SO_2 + H_2O \rightarrow Mg(HSO_3)_2$. Conditions in these scrubbers are harsh (temperatures > 60 °C, pH in the range of 4-7, high ionic strength). Together with complex interactions between gas phase and liquid media, these conditions lead to the formation of insoluble salts, which reduces the recovery efficiency and causes unscheduled downtimes and increased maintenance costs. Hence, an improved process

understanding is needed to counteract these precipitations. However, the harsh environment proves challenging, as most available chemical process probes cannot withstand the process conditions, and those that do quickly degrade in industrial practice.

We demonstrate the use of Raman spectroscopy to address this challenging PAT task. Raman spectroscopy can be performed in-line in a non-invasive and/or non-contact way. We show that Raman is fit for purpose, by being able to monitor process parameters such as free SO₂, bound SO₂, total SO₂ and monosulfite in real-time – parameters that are crucial for process control, and presently typically determined by sampling and titration. Furthermore, we outline how this can be used to help build and validate a digital twin of the scrubbers and use that as a most efficient and effective tool for process optimization.

22PMA07: Advances in the Analysis of Nanomaterials for Health

Chair: Zahra Rattray

(PMA-07.1) Improving Sensitivity of Detection using Magnetic Particles Coupled with SERS

Nikesh N. Patel¹, Duncan Graham², Karen Faulds², Stacey Laing¹; ¹*University of Strathclyde*, ²*The University of Strathclyde*

Rapid, selective and sensitive detections of analytes by merging magnetic particles with SERS detections.

Improving the sensitivity of analytical detection techniques is an area of significant importance when detecting analytes of interest. Incorporating improved methods within current technologies allows for better limits of detection to be achieved using existing methods of analysis. For instance, the use of magnetic beads (MBs) has shown extensive potential within medical and biological applications, due to their ability to separate specific entities within a complex matrix. Surface enhanced Raman scattering (SERS) is a highly sensitive and selective technique in bioanalysis; yet literature in applying both magnetic properties and SERS detection using a single bead is limited. The aim of this research was to assess the feasibility and performance of merging the sensitivity of SERS with the advantages of magnetic separation.

To achieve SERS, a Raman-active molecule and a roughened metal surface are required. Methods of functionalising the surface of MBs with gold nanoparticles (AuNPs) and Raman reporters were assessed, so that an optimum SERS signal could be achieved. This approach investigated the use of encapsulated gold nanotags attached to the surface of magnetic microparticles. The nanotags provided intense SERS signals and their addition to the surface

of magnetic beads gave a reproducible method of achieving stable SERS-active particles with inherent magnetic properties. The resulting beads also have the ability to be functionalised with a wide variety of biomolecules, allowing for targeted detection in biological assays.

SERS-active MBs can be used in a variety of different ways. One method of utilising the particles is to functionalise the beads with biomolecules such as DNA aptamers or antibodies, such that analytes of interest can be specifically captured on a surface and quantified using SERS. Another methodology is to apply the functionalised SERS-active MBs in a sandwich assay format, allowing the amplification of SERS signals upon biomolecule recognition, to identify and quantify analytes of interest. This approach is being investigated for application in antimicrobial resistance studies, where nanoparticles are functionalised with novel DNA aptamers specific to antibiotics, for the fast, sensitive and selective detection and quantification of abused antibiotics in waste water supplies.

(PMA-07.2) In Situ Real Time Monitoring of Emulsification and Homogenization Processes for Vaccine Adjuvants

Nicole Rabovsky¹, Joseph P. Smith¹; ¹*Merck & Co*

Process analytical technology uncovers vital process information during the formation of vaccine adjuvants

Adjuvants are commonly employed to enhance the efficacy of a vaccine and thereby increase the resulting immune response in a patient. The activity and effectiveness of emulsion-based adjuvants has been heavily studied throughout pharmaceuticals; however, there exists a lack in research which monitors the formation of a stable emulsion in real time. Process analytical technology (PAT) provides a solution to meet this need. PAT involves the collection of in situ data, thereby providing real time information about the monitored process as well as increasing understanding of that process. Here, three separate PAT tools – optical particle imaging, in situ particle analysis, and Raman spectroscopy – were used to monitor two key steps involved in the formation of a stable emulsion product, emulsification and homogenization, as well as perform a stability assessment. The obtained results provided new insights – particle size decreases during emulsification and homogenization, and molecular changes do not occur during either the emulsification or homogenization steps. Further, the stability assessment indicated that the coarse emulsion product obtained from the emulsification step is stable over the course of 24 hours when mixed. To the best of our knowledge, this is the first report of an analytical methodology for in situ, real time analysis of emulsification and homogenization processes for vaccine adjuvants. Using our proposed analytical methodology, an improved understanding of emulsion-based vaccine adjuvants can now be achieved, ultimately impacting the ability to develop and deliver successful pharmaceuticals.

(PMA-07.3) A Breakthrough in Inline Nanoparticle Sizing and Process Control for Nanosuspension Manufacturing

Rut Besseling¹, Carl Schuurmans¹, Raquel Arribas Bueno¹, Michiel Hermes¹, Remy van Tuijn¹, Ad Gerich¹; ¹*InProcess-LSP*

Novel Spatially Resolved DLS uniquely allows realtime inline nanoparticle sizing of turbid and flowing nanosuspensions

Nanodispersions take an increasingly prominent role in various industries. In their development and manufacturing ('bottom-up' or 'top-down'), inline monitoring of size characteristics during processing can give strong advantages both at R&D level, during upscaling and in controlled manufacturing. Regulatory bodies in pharma also emphasize the need for 'Process Analytical Technology (PAT)' for nano(medicine) dispersion processes, but so far implementation has been lacking primarily due to limitations of current measurement technologies -such as traditional Dynamic Light Scattering (DLS)- for characterization of nanosuspensions over large turbidity range and in process flows.

We recently introduced the NanoFlowSizer (NFS) with novel Spatially Resolved Dynamic Light Scattering (SR-DLS) technology. The NFS is a fully non-invasive PAT system that overcomes the limitations for inline measurement of traditional DLS methods. In particular, SR-DLS is based on Fourier Domain broadband (low coherence) interferometry, which resolves scattered light and its fluctuations with a resolution of a few micron over a few mm depth in the sample. After highlighting the technology, we describe several breakthrough examples of inline real-time monitoring using this instrument, both of bottom up and top-down nano-suspension manufacturing methods. These will include the continuous monitoring of a liposome extrusion process and nano-emulsion homogenization process, including process control based on the instruments inline size characterization. The NFS is specifically suited to monitor (cycled) processes requiring a specific endpoint and for continuous nano-dispersion manufacturing technologies. We discuss the added value of integrating PAT in such processes with emphasis on process/quality control.

(PMA-07.4) On/Off Fluorescent Detection of Cancer Biomarker in Cancer Cells

Sulayman A. Oladepo¹; ¹*King Fahd University of Petroleum and Minerals*

New cheaper and simpler smart probe system for direct detection of cancer biomarker in cells

This work presents a new mix-and-read homogeneous assay system consisting of a fluorescent smart probe for the on/off detection of miR-21 in MCF-7 cancer cell line. The smart probe was fully characterized and its thermal transition profiles proved its hairpin

structure and its sequence-specific hybridization with the intended miRNA target, while discriminating against mismatch sequences. More importantly, the new smart probe system was tested for the direct detection of miR-21 target in crude extractions from MCF-7 cancer cell line in a 96-well plate. The assay was able to detect the cancer target by producing high fluorescence signals while discriminating against cells that did not contain miR-21 target. The assay was carried out at room temperature and without the use of amplification enzymes. Thus, this new smart probe system constitutes a simple, homogeneous, mix-and-read detection technique that can provide reliable diagnostics of miR-21 cancer biomarker at room temperature and without the use of amplification enzymes. In addition, with the use of specific smart probes for each cancer biomarker of interest, the new method is capable of multiplex analysis of various cancer biomarkers in a 96-well plate.

22RAM11: Raman Spectroscopy for Food Security

Chair: Royston Goodacre

(RAM-11.1) Understanding the Impact of Adjuvants on Pesticide Persistence and Penetration in Fresh Produce using Surface-Enhanced Raman Mapping

Xinyi Du¹, Lili He¹; ¹*University of Massachusetts Amherst*

The widespread use of pesticides in agriculture has led to increased social concern about the potential hazards of pesticide residues on and in food. Therefore, it is significant to monitor and control these residues on and in food. In this study, we aimed to investigate the impact of different commercial adjuvant products, including non-ionic surfactant, oil, acidifier and buffer, on the persistency of pesticide active ingredients (AI) applied on fresh produce. Two model pesticide AI, thiabendazole (systemic) and phosmet (non-systemic), and two model fresh produce (spinach and apple) were tested. Surface-enhanced Raman spectroscopic (SERS) mapping was used to analyze the presence and distribution of pesticide AI with and without adjuvants on and in fresh produce in situ and real time. The results demonstrated that the adjuvants we tested in this study improved the spreading of pesticide AI on produce surfaces. However, they had no significant impact on the efficacy of sodium bicarbonate soaking to remove pesticides after short-term or long-term exposure on the fresh produce surface. Furthermore, the ethoxylate non-ionic surfactants containing formulation tested in this study had different penetration tendency compared with pure pesticide AI.

(RAM-11.2) Detecting Microplastics in Plastic Teabag Leachates via Infrared and Raman Spectroscopy

Cassio Lima¹, Royston Goodacre¹; ¹*The University of Liverpool*

Plastics have become indispensable products of daily life in many sectors. Currently, the majority of plastics used in everyday life are synthetic polymers derived from petroleum-based sources, which takes hundreds of years to decompose. The mismanagement of plastics has led to a significant environmental burden of growing concern as plastics have been spotted on different places around the globe from the poles to the tropics including seas, oceans, mountains, and urban environments. Recent reports have shown the negative impacts due to the ingestion of plastics in a wide range of organisms, especially smaller

fragments such as nano/microplastics, and there is an increasing concern about their effects on human health as recent studies have reported the presence of nano/microplastics in daily life consumer products such as bottled water, teabags, baby bottles, food containers, among others. Several analytical techniques have been proposed to study nano/microplastics, however, no single approach has been successful on fully describing their complexity. Here, we discuss the applicability and limitations of infrared and Raman spectroscopy to characterize nano/microplastics from plastic teabag leachates based on the ability of both methods to provide information about size, morphology, and chemical complexity.

(RAM-11.3) Non-Invasive Plant Genotyping and Identification of Pathogen Resistance Using Raman Spectroscopy

Dmitry Kurouski¹, Dmitry Kurouski¹; ¹*Texas A&M University*

Identification of plant species and their varieties for distinct phenotypic or genotypic traits whether using visual characterization or laboratory analysis requires substantial expertise, time, and resources. A less subjective and more precise method is needed for identification of peanut germplasm throughout the value chain. In this talk, I will demonstrate the use of Raman spectroscopy (RS), a non-invasive, non-destructive technique, in peanut phenotyping and identification. Our recent findings show that RS can be used for highly accurate peanut phenotyping via surface scans of peanut leaves and the resulting chemometric analysis: On average >90% accuracy in identification of plant varieties and breeding lines can be achieved. Our results also suggest that RS can be used for highly accurate determination of nematode and fungal resistance and susceptibility of those breeding lines and cultivars. Specifically, nematode-resistant peanut cultivars can be identified with 92% accuracy, whereas susceptible breeding lines were identified with 81% accuracy. Similar accuracies of identification of plant resistance to fungi were also obtained in both greenhouse and field experiments.

(RAM-11.4) Raman Spectroscopy as a Tool for Understanding Oil or Fat Quality in Food Products

Karen A. Esmonde-White¹, Mary Lewis¹, Michael Donahue¹, Ian Lewis¹; ¹*Endress+Hauser*
Lab-to-process Raman for measuring oil quality in a variety of foods

Many natural and produced foods contain oils or fats, and their presence affects the product shelf life and sensory attributes. Analysis of oils or fats in these foods can be carried out using wet chemistry, chromatographic, or spectroscopic techniques. Molecular spectroscopic techniques of near-infrared, infrared, or Raman have the benefit of providing a non-destructive chemical and molecular structure analysis with the ability to measure oil or fat quality directly in a process or in the laboratory. Raman spectroscopy is a molecular spectroscopy technique that is highly specific, can measure in aqueous systems, and provides a multi-attribute measurement in a single probe. We provide an overview of the information provided by a Raman spectrum, including the presence of cis or trans isomers, fatty acid saturation, and polymorphic stability. An application example of Raman

spectroscopy in dark and milk chocolate will be presented to showcase Raman's capability to measure qualitative and quantitative quality aspects of fats in a complex and highly fluorescent matrix. Our initial feasibility studies showed initial markers of cocoa butter quality that support additional studies with more samples.

(RAM-11.5) High Throughput Microplastic Characterization Using Particle Correlated Raman Spectroscopy

Bridget O'Donnell¹, Eunah Lee¹; ¹*HORIBA Scientific*

Automation of microplastic measurements using particle correlated Raman spectroscopy for high throughput

Microplastics are ubiquitous, from drinking water to marine environments, sediments, and tissues. Characterization of microplastics can be time-consuming, particularly when they are hand-picked manually and characterized one at a time using a Raman microscope. Particle Correlated Raman Spectroscopy (PCRS) offers an automated method for microplastic characterization, from characterizing particle morphology through chemical identification for high throughput screening of microplastic samples. In this talk, the PCRS technique will be presented in addition to a discussion of sample handling and preparation of microplastic samples from various matrices in preparation for PCRS measurements.

22SPECIAL05: Regional Industrial Research

Chair: Gloria Story

(SPEC-05.1) Developing Complex Fluids in Microgravity

Matt Lynch¹, Thomas Kodger, William Meyer, Mark Pestak; ¹*Procter & Gamble*

A series of microscopic and macroscopic measurements of model colloidal gel systems has been done on the International Space Station (ISS), inspiring the development of transformative materials. The model system is composed of mixtures of sticky, micron-size colloid particles that is an analog of a commercial gel system. The unique microgravity environment has enabled the study of the systems without the complications of sedimentation; however, the application of small temperature gradients resulted in Soret forces that have enabled the study with very small gravitational effects. The seminar will share how these measurements are made and how they aid in develop complex fluids in microgravity.

(SPEC-05.2) Dissolution Recycling of Polyolefins Using Alkane Solvents

John Layman¹, John Layman¹, Dimitris Collias, Amy Waun; ¹*Procter & Gamble*

Consumers increasingly expect and demand sustainable products without trade-offs in performance or cost. Concerned companies, like P&G, have established long-term sustainability goals that include the use of large percentages of recycled resins in their products and packaging. To satisfy consumers' expectations and achieve companies' goals, P&G has developed a novel purification technology that converts contaminated recycled polyolefins (PP and PE) into virgin-like resins.

The dissolution recycling technology purifies recovered polyolefins using an alkane solvent at elevated temperature and pressure, and a combination of standard chemical engineering unit operations, such as liquid-liquid extraction, sedimentation, size exclusion and adsorbent filtrations, and devolatilization. These processes remove pigments, odor, volatile organic chemicals, and other organic and particulate contaminants and additives rendering a material with virgin-like color and appearance.

(SPEC-05.3) Cleaning Clothes in Space and Applications for Consumer Use

Mark R. Sivik¹, Will Shearouse, Kristi Niehaus, Steven Patterson; ¹*Procter & Gamble*

Currently, there are no viable processes to clean clothing in space. As space exploration and duration of space travel increases, there will be a need to have a viable laundry process for cleaning crew garments long term due to cost and mass of shipping clothing into space. For a 3-year mission, an estimated 460 lbs (210 kg) of clothing is required per crew member. In addition, water is also costly to ship making it a limited resource that needs to be recoverable. The detergent and process must also be compatible with closed loop systems and be fully degradable to allow for efficient water recovery. While these requirements are needed for future space missions, many of these constraints exist today and the need to have viable efficient laundering process will become more important as water scarcity continues around the globe. Therefore, a fully degradable detergent and laundering processes are being developed with NASA and P&G that are compatible with closed loop systems for use under constrained conditions to determine the viability of cleaning clothes on Lunar and/or Mars habitats. Learnings from this program and developments to date will be presented along with their application for consumers on Earth and towards P&G's Ambition 2030 sustainability goals.

(SPEC-05.4) Soft Material Characterization: Translating Clinical Magnetic Resonance Imaging Methods for Consumer Products Research

Nicole Westrick¹, Nicole Westrick¹; ¹*Procter & Gamble Co.*

At Procter & Gamble, we have enabled consumer product innovations with magnetic resonance imaging (MRI) through its application as an analytical tool to simulate consumer use experiences, provide mechanistic understanding of how products work, develop predictive models, and create visuals which bring our products to life for consumers. In this presentation, creative approaches for analyzing soft materials with 3D magnetic resonance elastography (MRE) will be shared. MRE is a new imaging technique to non-invasively quantify the biomechanical properties of soft tissues by visualizing propagating shear waves

with a modified phase-contrast MRI sequence. Shear stiffness is calculated, and the wave image, a visualization of shear wave motion in the tissue, is converted to an elastogram. While in clinical applications differences in mechanical stiffness are used to differentiate healthy and diseased tissue, we are interested in MRE's utility for characterizing shear stiffness in consumer product-relevant soft materials. We will present MRE shear modulus measurements for gelatin phantoms.

(SPEC-05.5) Dynamic Computed Tomography for Product Research and Development
Laura Wiley¹, Alex Doukas¹; ¹*Kinetic Vision*

SUMMARY

Advancements in design iteration techniques have been accelerating over the past three decades, and have largely focused on replicating final manufactured product behavior. Integration of Industrial CT, modeling & simulation, and rapid prototyping has enabled accelerated analysis of dimensionality and physics affecting a product's quality and performance. New apparatuses are being integrated to provide real-time, dynamic CT scanning yielding behavioral geometries and deformations collected during the X-Ray process. Dynamic scanning allows for expanded input metrics and enhanced validation across the product development lifecycle.

This presentation will focus on showing the increased speed of product development due to integration of dynamic 4D CT scanning, modeling & simulation, and rapid prototyping methods to validate complex product performance models. The speed of concept-to-market is rapidly increasing, with higher demands on faster and better product testing, inspection, and analysis. The presentation concludes that dynamic 4D behavioral scanning can not only significantly improve the validation of advanced simulation, it can also help drive better efficiency and accuracy in the production and evaluation processes.

Topics will include:

1. Introduction of CT scanning methodologies and their use cases
2. Development of modeling & simulation validation using CT data
3. Development of fixtures for dynamic scanning to assess product behavior
4. Advancements in rapid prototyping materials and methods

KEY WORDS: Dynamic scanning, rapid prototyping, modeling & simulation, product development, validated Modeling, material testing, CT, computed tomography, X-Ray, geometries, 4-D imaging.

22SPR02: Optical and Chiral Properties of Plasmonic Nanoparticles

Chair: Xingchen Ye

(SPR-02.1) Controlling Localized Plasmons via an Atomistic Approach

Nan Jiang¹; ¹*University of Illinois Chicago*

Chemical reactions such as bond dissociation and formation assisted by localized surface plasmons (LSPs) of noble metal nanostructures hold promise in solar-to-chemical energy conversion. However, the precise control of localized plasmons to activate a specific moiety of a molecule, in the presence of multiple chemically equivalent parts within a single molecule, is scarce due to the relatively large lateral distribution of the plasmonic field. Herein, we report the plasmon-assisted dissociation of a specific molecular site (C–Si bond) within a polyfunctional molecule adsorbed on a Cu(100) surface in the scanning tunneling microscope (STM) junction. The molecular site to be activated can be selected by carefully positioning the tip and bringing the tip extremely close to the molecule (atomistic approach), thereby achieving plasmonic nanoconfinement at the tip apex. Furthermore, multiple reactive sites are activated in a sequential manner at the sub-molecular scale, and different sets of products are created and visualized by STM topography and density functional theory (DFT) modeling. The illustration of site-selective activation achieved by localized surface plasmons implies the realization of molecular-scale resolution for bond-selected plasmon-induced chemistry.

(SPR-02.2) **Development and Characterization of Plasmonic Terahertz Sensors for Biological Analysis**

Santino N. Valiulis¹, Alexander S. Malinick², Quan Cheng²; ¹*University of California, Riverside*, ²*University of California Riverside*

Aluminum plasmonic chips that plasmonically respond to THz light used as sensors.

There has been a recent trend in terahertz (THz) sensing applications towards plasmonic nanomaterials and metamaterial surfaces that generate evanescent fields that amplify the low inherent response of THz spectroscopy. As THz spectroscopy has many advantages compared to that of the visible range frequently used with surface plasmon resonance (SPR), specifically the ability to amplify signal through coupling of the plasmonic resonant dip and absorption of specific bonds in the analyte of interest. This allows for differentiation in signal through detection of specific bonds that absorb in the THz range. Current work in development of THz sensors frequently relies on homebuilt spectroscopic instruments with usage explicitly designed for THz systems, which limits its applicability as a broader analytical technique. Our work involves taking advantage of instrumentation commonly available, an IR spectroscope, and developing a metamaterial chip that plasmonically resonates in the ranges available within IR instrumentation, 2-230 THz. Predictions of structures that would provide the needed resonance were explored using finite domain-time domain simulations (FDTD). These allowed us to optimize the metamaterial surface to determine the locations of the plasmonic peaks in the spectra, so that future coupling to analytes can be predicted, and the amplitude of the peaks can be improved. Based upon the FDTD simulations, a metamaterial

system is determined and a photomask is designed for usage in photolithographic techniques. FDTD simulations and fabrication is done with aluminum as the plasmonic material. Aluminum is chosen as the material for a variety of reasons, including high plasmonic activity, natural oxidation layer, and environmental availability. As we have recently demonstrated aluminum is highly effective as a sensitive plasmonic material that also eases cleanroom fabrication processes. The developed sensors were characterized using AFM and their sensitivity to refractive index is determined. In addition, their ability as a biosensor is explored, through using ganglioside detection with the ability to differentiate between them based off of their THz absorption signature as well as the ability to shift the plasmonic response.

(SPR-02.3) Field Enhancement Between the Single-Reflection ATR-FTIR and Plasmonic Surfaces

Li-Lin Tay¹, Nelson Rowell²; ¹*National Research Council Canada*, ²*NRC*

Additional field enhancement can be realized by combination of substrate, angle and ATR element

Attenuated total reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy is a versatile surface analytical technique used in many materials analysis applications. There are a number of different types ATR-FTIR configurations. It can be configured by coupling light into a trapezoidal prism with light bounces multiple times in the prism before exiting. Another configurations is through a hemispherical or triangular prism, with light coupled into the prism and reflected off in a single bounce. We will present experimental studies and simulation of plasmonic substrates with plasmonic resonance in the MIR range probed with a single bounce ATR configuration. Under such configuration, incident light is coupled through a high refractive index hemispherical Ge prism at variable angles that is controlled by a variable angle reflectometer. In this configuration, radiation with an electric field component normal to the surface (e.g. p-polarized light) experiences a significant enhancement. This leads to an increased optical absorption and improves the sensitivity of the molecules that are present at the interface. This improvement in spectroscopic sensitivity allows various surface properties to be investigated, including the identification of sub-monolayer molecular species. Our measurement showed that the enhancement is further increased by the presence of the plasmonic substrate. In this presentation, we will show that a discontinuity of the field normal to the ATR crystal contributes to the field enhancement in the lower index thin film which can results in two-orders of magnitude increase in the sensitivity of single bounce ATR-FTIR. We will present the study of sub-monolayer undecylenic acid self-assembled on Si(111) surface and thiolated film on patterned nanowire Au surfaces.

(SPR-02.4) Probing Infrared Plasmons with Electron Energy Loss Spectroscopy

Jon P. Camden¹; ¹*University of Notre Dame*

This work explores coupling infrared plasmons with molecular vibrations and phonons at the nanometer scale.

Waste heat generation is an unavoidable consequence of work performed across all areas of human activity, including industrial processes, electricity generation, transportation, and information processing. It is estimated that 20 – 50% of commercial energy input is lost to the environment as waste heat; therefore, the ability to reclaim even a fraction of this lost heat represents a major opportunity to improve energy efficiency. Such improvements would be especially meaningful in limited resource environments, such as in spacecraft or off-grid locations.

Towards this end, rationally designed infrared (IR) plasmonic and phononic materials offer the potential to harness and direct the flow of energy from thermal sources, but controlling the energetic location of their responses is challenging yet necessary to align with those thermal sources. In this research, we investigate a promising new class of semiconductor materials that offer robust, wavelength-tunable mid-IR plasmonic excitations through introduction or removal of free charge carriers. This spectral tunability lies above and beyond the tunability achieved through the hybridization of multiple material units. Specifically, using energy-monochromated scanning transmission electron microscopy (STEM) electron energy-loss spectroscopy (EELS), we spatially and spectrally map the mid-IR plasmonic excitations of individual indium tin oxide (ITO) nanocrystals as a function of Sn⁴⁺ composition. Both nanocrystal monomers and dimers are studied, with the latter showing enhanced local fields that are surprisingly comparable to those of the more familiar noble metals in the visible part of the spectrum. With the tunability of their carrier density and enhanced local fields of their hybrid modes, ITO nanocrystals offer a promising new class of materials that we are now coupling to other infrared active materials and thermal heat sources and imaging their interactions via STEM-EELS.

(SPR-02.5) Ligand Rotational Isomer Effects on Optoelectronic Properties of Gold Clusters

Christopher J. Ackerson¹, Gowri Udayangani Kuda-Singappulige, Christopher J. Ackerson¹, Christopher Hosier, Ian Anderson, Christine Aikens; ¹*Colorado State University*

The crystal structures of 4 ligand-rotational isomers of $\text{Au}_{25}(\text{PET})_{18}$ are presented. Two new ligand-rotational isomers are revealed, and two higher-quality structures (allowing complete solution of the ligand shell) of previously solved $\text{Au}_{25}(\text{PET})_{18}$ clusters are also presented. One of the structures lacks an inversion center, making it the first chiral $\text{Au}_{25}(\text{SR})_{18}$ structure solved. These structures combined with previously published $\text{Au}_{25}(\text{SR})_{18}$ structures enable an analysis of the empirical ligand conformation landscape for $\text{Au}_{25}(\text{SR})_{18}$ clusters. This analysis shows that the dihedral angles within the PET ligand are restricted to certain observable values, and also that the dihedral angle values are interdependent, in a manner reminiscent of biomolecule dihedral angles such as those in proteins and DNA. The influence of ligand conformational isomerism on optical and electronic properties was calculated, revealing that the ligand conformations affect the nanocluster absorption spectrum, which potentially provides a way to distinguish between isomers at low temperature.

Poster Presentations

Wednesday Poster Session - AES

(Wed-P01) **Electrokinetic lithography to engineer the collagen fiber microarchitecture**

Adrian Lomeli-Martin¹, Adeel Ahmed¹, Mehran Mansouri¹, Vinay V. Abhyankar¹, Blanca H. Lapizco-Encinas¹; ¹*Rochester Institute of Technology*

It is a well-known fact that cells can both sense and respond to mechanical signals transmitted through the extracellular matrix (ECM). The extent to which motility and long-range cell communication are impacted by local breaks in the collagen fiber alignment remains unclear. To both provide insight into these queries and to expand upon current type 1 collagen (COL 1) fabrication capabilities, the present work aims to implement a new biomaterial engineering approach coined electrokinetic lithography (EKL). This work is built on the hypothesis that the motion of charged particles directed by controlled electric fields (EK) will result in a localized disruption of COL 1 fiber alignment and, in turn, would allow the formation of tunable discontinuities in the gel. These discontinuities would enable us to explore if and how breaks in the ECM impact cell motility and long-range communication.

Acknowledgments:

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(Wed-P02) **Towards an Understanding of AC-Electrokinetic Effects in the Separation of Nanoplastics**

Shulin Bu¹, Alexandra Ros¹; ¹*Arizona State University*

Microplastics have become an emerging threat to terrestrial and marine systems as they contaminate oceans, lakes and soils impacting biodiversity and the ecosystem. Referred to small plastic particles with diameter < 5 mm, microplastics are now recognized as a significant threat to the environment and humans. Accumulation of microplastics in human

bodies may cause potential health issues such as hypersensitivity and acute response. Nanoplastics with dimensions $< 1 \mu\text{m}$ pose a potential high risk to animals and humans, as these particles have been found in blood and other body fluids. However, there are limited methods to detect and quantify nanoplastics.

Here, we propose studying the AC electrokinetic effects of nanoplastics in body fluids to develop detection and analysis tools that will help elucidate nanoplastics found in organisms and relate to potential human health issues. We focus on two main effects: dielectrophoresis (DEP) and AC-induced electroosmosis (ACEO). DEP is the movement of polarizable particles in a non-uniform electric field and ACEO refers to the flow induced by AC electric fields. We selected well-characterized polystyrene (PS) particles and subjected them to varying experimental conditions such as the suspended medium ionic strength and the applied frequency in insulator-based DEP devices. We hypothesize that nanoplastics occur in body fluids coated with high abundant biomolecules and chose the protein bovine serum albumin (BSA) as a model to study to influence of protein coating on PS particles. To gain insight into the low-frequency dielectric properties, coated and non-coated PS beads were characterized using dynamic light scattering revealing a decrease in Zeta potential upon protein adsorption. While PS particles have recently shown negative DEP behavior, we also investigate the changes in DEP properties based on the variation in Zeta potential and suspended medium ionic strength. We further report the regimes of unique DEP trapping behavior as well as the combination with ACEO effects for various PS particle sizes $< 1 \mu\text{m}$. Our fundamental study will aid in the understanding of the low-frequency AC-electrokinetic behavior of nanoplastics and contribute to developing highly effective analytical tools for pre-concentration, analysis and separation of nanoplastics.

(Wed-P03) Using Deep Eutectic Solvents as Reaction and Separation Media for Capillary Electrophoresis

Karen S. Campos¹, Jessica Torres¹, Shreeya Venkatesan¹, Christopher R. Harrison¹; ¹*San Diego State University*

Deep eutectic solvents (DES) are non-aqueous homogeneous binary mixtures, there exist nearly endless combinations of hydrogen bond donors and hydrogen bond acceptors that can yield DES. Hallmarks of DES are their liquid state below the melting points of either of the individual components, solubility characteristics that vary with their composition, and low volatility. Many DES have been shown to be effective alternative solvents for situations where volatile non-aqueous solvents are undesirable. DES have been used for extractions, nanoparticle production, and biofuel processing. The use of DES in separation sciences has been almost only as minor additives to buffers. This work will present the first applications of DES in the pre-separation labeling process, and as the primary component of a CZE separation buffer.

We will present our evaluation of the efficacy of the DES ethaline, a 2:1 molar ratio of ethylene glycol and choline chloride, as the reaction solvent for the fluorescent labeling reaction of amino acids for CE-LIF analyses. A number of different fluorescent reagents, with varied bonding chemistries, have been tested and FITC proved to be the most effective. Under the optimized reaction conditions, the fluorescent amino acids can be detected using

CE-LIF concentrations as low as 50 nM.

The use of ethaline in CE buffers has been restricted to that of a minor additive largely due to the ionic composition of the DES. With the salt choline chloride composing a third of the solvent, the pure DES is far too conductive to be used as a CE buffer. Our solution to this is to use an analog DES, where the choline chloride is replaced with non-ionic 2-dimethylaminoethanol (DMAE). The new DES has high resistivity but also has the ability to deprotonate the capillary surface, resulting in a weak EOF, making it a viable CE BGE. We will present our evaluation of the viability of this DES for electrophoretic separations, and the impact of the addition of water to the BGE.

(Wed-P04) Solvent Mediated Forces in Protein Dielectrophoresis

Michael Sauer¹, Mark A. Hayes¹, Matthias Heyden¹; ¹*Arizona State University*

Dielectrophoresis (DEP) is a well-established technique to manipulate micrometer-sized particles. Standard continuum theory predicts negligible effects for nanometer-size proteins, but a large body of experimental evidence captured over many years, in disparate experimental conditions and by many separate researchers show significant and distinct effects consistent with dielectrophoretic behaviors. Molecular scale details of proteins, such as the static dipole moment and protein-water interactions, are unaccounted for by the standard continuum theory suggesting an opportunity to explore these effects theoretically.

Previous theoretical studies have shown that the DEP force can be described by free energy changes in the system. Computational studies based on classical molecular dynamics (MD) simulations have shown that the free energy change associated with protein dipole polarization (field-mediated interactions) and the free energy change associated with protein-water interactions (solvent-mediated interactions) both contribute to the overall DEP force. In these calculations, the favorable free energy contribution from field-mediated interactions dominates. This leads to an overall positive DEP force that is highly dependent on protein-electric field interactions.

However, these simulations are unable to explain experimental evidence supporting negative DEP forces on proteins. To continue to explore differences between theoretical predictions and experimental observation, we extend computational studies to large proteins with small dipole moments. For large proteins, an increase in the protein-water interface leads to an increase in individual protein-water interactions. For proteins with small dipole moments, interactions with the electric field will diminish.

We predict that for large proteins, solvent-mediated forces will become an important contributor to the overall DEP force. In addition, unfavorable (positive) changes in the solvation free energy describe a negative solvent-mediated DEP force. If the solvent-mediated force is greater than the field-mediated force, solvation will also describe an overall negative DEP force.

Using atomistic MD, changes in the protein solvation free energy are spatially resolved and the magnitude of these protein-water interactions are compared to the magnitude of the

protein-electric field interactions. Using this free energy framework, the effective dielectrophoretic force caused by solvation and protein polarization is reported, providing potential explanations for the experimental observation of negative protein dielectrophoresis.

(Wed-P05) Isolation, Enrichment, and Recovery of Microparticles using Dielectrophoresis

Jared P. Smithers¹, Mark A. Hayes¹; ¹*Arizona State University*

Dielectrophoresis (DEP) has been widely proven as a viable high-resolution separation technique for biological samples like bacteria, proteins, and viruses. In our application, DEP involves the use of an applied voltage to a microfluidic channel with sequentially changing, constrictive insulating features, known as gates. As a result, non-uniformities in the electric field are created inducing DEP. At a specific voltage, the electrophoretic and electroosmotic forces propelling the particles through the channel become balanced with a counteracting DEP force which causes unique trapping formations at gates that match the properties of the analyte. Highly specific analytes are settled into discrete zones of the channel within the insulating microdevice.

Due to the location of the trapping zones within the device, and the fact that particles can only be trapped with the presence of an electric field, recovery of the separated particle bolus has not been successfully achieved. The geometry of the device limits elution techniques and they cannot be implemented. In this work, we focus on the addition of microfluidic side channels near the trapping gate of the microfluidic channel to recover the isolated enriched bolus of trapped particles. This can be achieved using a voltage scheme that sequentially traps the particles near or at a specific gate by the main channel electric field and then redirect the electric field through the side channels, inducing electroosmotic flow which sweeps the particles to an accessible external port for recovery. In turn, separated analytes can then be effectively transferred and coupled to other information-rich detection systems to confirm the identity of the analyte and the effectiveness of the DEP separation technique. Achieving efficient recovery of analytes separated by DEP would help bridge this emerging separation technique into more conventional analytical chemistry applications like purification/pre-concentration treatments, rapid diagnostic testing of pathogens/virus, and as a robust detection system of its own.

(Wed-P06) High-Frequency Dielectrophoresis Reveals Distinct Bioelectric Signature of Cancer Cells with Varying Ploidy and Nuclear Size

Josie L. Duncan¹, Mathew Bloomfield¹, Vahid Farmehini², Nathan Swami², Daniela Cimini¹, Rafael Davalos¹; ¹*Virginia Tech*, ²*University of Virginia*

Whole genome doubling (WGD) occurs in over 30% of human tumors and is associated with poor prognosis. WGD is the result of failed cell division, which leads to the formation of cells with tetraploid (4N) chromosome content. We hypothesize that chromosome content, consisting of negatively charged DNA molecules, will affect the intracellular dielectric

properties and is detectable by high-frequency dielectrophoresis (HF-DEP). HF-DEP, to our knowledge, has never been used to study the dielectric behavior of isogenic cells with varying ploidy. To test our hypothesis, we used diploid (2N) colorectal cancer cells to generate tetraploid (4N) clones that varied in cell and nuclear size, but not DNA content. We then performed HF-DEP at frequencies between 10-100MHz using a gold interdigitated electrode array. We found that the 2N cells and small and large 4N clones showed distinct signatures at a frequency range of 40-60MHz. While the signatures of the small 4N clones suggested an increase in nucleoplasm conductivity, the signatures of the large 4N clones suggested a decrease in nucleoplasm conductivity when compared to the 2N cells. This shift indicates that the detected differences can be attributed to charge density rather than charge quantity of nuclear DNA.

Our data also showed a significant drop-off in fraction of trapped cells beyond 40MHz. Using a COMSOL model, we found that as the frequency increases, the impedance decreases, resulting in decreased voltage reaching the sample. At 40 MHz, the voltage reaching the cells drops to 1Vpp, suggesting a critical value for trapping cells on this device. At higher frequencies, trapping occurs at progressively decreasing rates and approaches zero at frequencies above 70MHz.

HF-DEP is a useful tool in studying ploidy in subpopulations, but the change in impedance at high frequencies must be considered before these populations can be well-characterized. The bioelectric signatures of 4N cells depends on the density of DNA within the nucleoplasm, rather than just an increase in charge quantity from doubling DNA content. Further, device performance is greatly degraded at high frequencies due to impedance losses, making it difficult to verify characteristic frequencies for each cell-type above 70MHz.

(Wed-P07) Biovariability Of Single Bacteria Isolate Measured With Label-free Insulator-Based Dielectrophoresis

Hoai T. Nguyen¹, Mark A. Hayes¹; ¹*Arizona State University*

Biophysical measurements of bacterial cells can be used for diagnostics, however, the open question of natural within-species biodiversity as reflected in variance of biophysical signature needs to be addressed.

Identification of the infecting bacteria and its antimicrobial susceptibility remains a significant challenge for treating physicians to issue appropriate treatment. The complexity of identification does not stop at the separation of strains, but also at the biovariability in a single strain or isolates, such as activities of genes (genomics), proteins (proteomics), metabolites (metabolomics), and cell phenotypes (phenomics). The existing techniques for phenotype quantification are culture-based, genomics-based, advanced imaging (Scanning Electron Microscopy, Transmission Electron Microscopy, and their auxiliaries), or target-specific fluorescent tagging. All these techniques have limitations such as time-consuming and non-specific (culturing), costly instruments and trained operators (genomics and imaging), or multiple highly specific fluorescent probes.

A promising alternative is microfluidic biophysical separation. Specifically, bacterial dielectrophoresis (DEP) offers high-resolution separation leading to identification in a short period of time (seconds). In this technique, trapping (or capture) of bacterial cells occurs when the voltage is applied to the microdevice, where target cells are immobilized in a constriction within the microchannel. Onset potential is the lowest potential at which capture is observed, and it is dependent of the ratio of electrokinetic (electrophoresis and electroosmosis) and dielectrophoretic mobilities, or electrokinetic mobility ratio (EKMr). This ratio reflects differences in bacterial biophysical properties between strains, including, but not limited to size, charge and charge distribution, surface functional groups and inner structures, bacterial viability, and metabolism. Published works have focused on fluorescent-tagged bacterial cells, which prolongs the experimental scheme and compromises the integrity of clinical samples. In this presentation, a label-free capture of bacterial cell *Staphylococcus epidermis* ATCC 35984, an isolate of clinical importance with fully sequenced genomes is shown. The onset voltage was determined and the quantification of biovariability is reflected in the EKMr measurements for a single strain of pathogen. The effort will lay the foundation in sample preparation, data processing, and validation for other bacterial isolates.

(Wed-P08) **Nonlinear Electrokinetics of Non-spherical Particles**

Olivia Ernst¹, Alaleh Vaghef Koodehi¹, Blanca H. Lapizco-Encinas¹; ¹*Rochester Institute of Technology*

The electrokinetic migration of particles and cells is dependent on their particle zeta potential (z_p) and their nonlinear electrophoretic mobility ($\mu_{EP}^{(3)}$), which can be determined through particle image velocimetry (PIV). The particle zeta potential refers to the surface charge of a particle/cell which directly determines the linear electrophoretic mobility. The E_{EEC} , a newly identified parameter [1], is where all electrokinetic effects exerted on a particle are in equilibrium, resulting in a net particle migration of zero. By employing PIV experiments carried out high electric field, it is possible to determine the E_{EEC} parameter, from which the $\mu_{EP}^{(3)}$ mobility of a particle can be estimated. This study aims to develop correlations between particle/cell zeta potential, E_{EEC} , and $\mu_{EP}^{(3)}$ as functions of particle/cell shape and size. Electrokinetic parameters can be then estimated by employing these correlations, saving substantial experimental time and effort. The data obtained from this study can be used to design electrokinetic separation processes of microparticles and microorganisms [2]. Preliminary results showed differences in particle/cell properties based on shape and size.

Acknowledgments:

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(Wed-P09) Trapping and Finite Element Analysis of Fluorescently-Tagged Gold Nanoparticles via Gradient Insulator Dielectrophoresis

Alex Ramirez¹, A K M Fazlul Karim Rasel¹, Sean L. Seyler¹, Mark A. Hayes¹; ¹*Arizona State University*

Gradient insulator dielectrophoresis (DEP) induces controlled particle movement by altering the respective forces of electroosmotic flow, electrophoresis, and DEP in a microfluidic device. For over two decades, such devices have demonstrated the ability to separate a large variety of analytes including cells, proteins, viruses, and more. The DEP force's (f_{DEP}) contribution to movement of micron-sized particles is well described by its classical equation, but the same cannot be said for the movement of nanoscale particles. Although DEP separations with nanoscale bioparticles can easily be found in the literature, these types of particles are inherently too variable to be used as a refinement for the classical f_{DEP} theory. This work used 10 nm FITC-tagged gold nanoparticles (AuNPs) as ideal analytes that were separated in a microfluidic device, the experimental results were then modeled via finite element analysis. Preliminary modeling using the classical f_{DEP} equation has indicated that f_{DEP} exerted on the AuNPs is 3-4 orders of magnitude higher than anticipated. These results represent initial efforts to approximate classical f_{DEP} 's inaccuracy for nanoscale particles.

(Wed-P10) Dielectric Characterization of Babesia Bovis using the Dielectrophoretic Crossover Frequency

Raphael O. Oladokun¹, Soumya Srivastava¹; ¹*West Virginia University*

Coinfection with the tick-transmitted pathogen *Babesia spp.* is becoming a serious health problem because of the erythrocyte invasion through *Ixodes scapularis* tick. The transmission of this protozoan by blood transfusion often results in high morbidity and mortality in recipients. A novel way to detect parasitized erythrocytes is by utilizing dielectrophoresis, an electrokinetic technique on a microfluidic platform to improve the diagnostics of *Babesia spp.* The difference in the dielectric properties of *Babesia spp.* infected erythrocytes vs. healthy erythrocytes were exploited to design a fast and cost-effective diagnostic tool. One crucial factor for a successful diagnostic platform via dielectrophoretic separation is the dielectric characterization of *Babesia* infected erythrocytes, which is investigated in this paper. The influence of medium conductivity and erythrocytes phenotype and genotype over the first crossover frequency (f_{co1}) are used to quantify the dielectric properties of the infected cells. A sigmoidal curve was plotted via curve fitting of the single-shell model, which has been proven to be appropriate for parasitized cell populations in which considerable cell geometry variation occur. The difference in these curves is relevant for separation of cells population.

Microliters of sample and reagent were used throughout this experiment; the scale, results obtained, and simplicity of the system often makes it very suitable to control experiment and do replications in babesiosis disease diagnosis.

Wednesday Poster Session - RAMAN

(Wed-P11) Vis-NIR Spectroscopy and Machine Learning Methods for Discrimination of Transgenic Canola (*Brassica napus* L.) and their Hybrids with *B. rapa*

Soo-In Sohn¹, Subramani Pandian¹, Young-Ju Oh², Hyeon-Jung Kang¹, Eun-Kyoung Shin¹, Senthil Kumar Thamilarasan¹, Tae-Hun Ryu¹, Woo-Suk Cho¹, Youn-Sung Cho¹, Tae-Sung Park¹; ¹*National Institute of Agricultural Sciences*, ²*Institute for Future Environmental Ecology Co., Ltd.*

In recent years, the fast development of genetically modified (GM) technology has raised distresses about the safety of GM crops and foods for human health and the ecological environment. Gene flow from GM crops to other crops, especially in the Brassicaceae family, might pose a risk to the environment due to their invasiveness. Therefore, finding reliable, rapid, and low-cost methods to detect and monitor the presence of GM crops and crop products is important. In this study, we used visible near-infrared (Vis-NIR) spectroscopy for the effective discrimination of GM and non-GM *Brassica napus*, *B. rapa*, and F1 hybrids (*B. rapa* X GM *B. napus*). Primarily, Vis-NIR spectra were collected from the plants, and the spectra were preprocessed. A combination of different preprocessing methods and various modeling approaches was used for effective discrimination. Among the different combinations, the Savitzky-Golay and Support Vector Machine combination was found to be an optimal model in the discrimination of GM, non-GM, and hybrid plants with the highest accuracy rate (100%). The use of a Convolutional Neural Network with Normalization resulted 98.9%. Later, phenolic acid concentration among the different plants was assessed using GC-MS analysis. Partial least squares regression analysis of Vis-NIR spectra and biochemical characteristics showed significant correlations in their respective changes. The results showed that handheld Vis-NIR spectroscopy combined with chemometric analyses could be used for the effective discrimination of GM and non-GM *B. napus*, *B. rapa*, and F1 hybrids. Phytochemical composition analysis can also be combined with the Vis-NIR spectra for effective discrimination.

(Wed-P12) In situ Raman spectroscopy Monitors the Corrosion of Mild Steel in a Salt Fog Chamber

Dieter Bingemann¹, Arie Bleij², Maria Ponomareva², Markus Nadlinger², Gabriela Schimo-Aichhorn², Gerald Luckeneder³, Gerald Haslehner³, Pierluigi Bilotto²; ¹*Wasatch Photonics*, ²*CEST Kompetenzzentrum für elektrochemische Oberflächentechnologie GmbH*, ³*voestalpine Stahl GmbH*

The global cost of corrosion imposes a significant burden on society, with safety and environmental consequences in addition to its direct impact. One challenge to its management is that the process of corrosion is complex, and common experimental batch processing techniques provide only limited information. The neutral salt spray test, a standardized test

used for the evaluation of the corrosion resistance of steels and coatings, creates an aggressive environment not suitable for many analytical methods. Here we report the use of in situ Raman spectroscopy to detect the formation, growth, and evolution of corrosion products on metal surfaces, monitoring a mild steel plate in alternating salt fog and dry atmospheric conditions over a period of eight days to identify and track key corrosion products. Via Raman spectra from the literature, we identify as key corrosion species iron oxy-hydroxides forming during the wet phase, converting to Iron(III) oxide and Fe_3O_4 during the dry phase. While Fe(III) oxide converts back to oxy-hydroxides during the next wet phase, the stable Fe_3O_4 remains as an oxide even under wet conditions. As a minor species we also find iron carbonate during the initial dry phase. Assisted by MCR-ALS analysis and online weighing of the sample, we determine the time scales of the conversions and find that the chemical conversion of iron oxyhydroxides to oxides takes several hours and hence much longer than the physical drying of the corroded surface. Together, these results pave the way for real-time online observation of corrosion inside a salt fog chamber to obtain chemically relevant information with a single, compact apparatus.

(Wed-P13) Super-resolution Surface Enhanced Raman Imaging of Protein Receptors in Cells

Abigail E. Smith¹, Zac D. Schultz¹; ¹*The Ohio State University*

Visualization of cells and their components has been a common practice in laboratories for centuries; however, this practice alone is limited to imaging on a large scale and does not provide information on the molecular level. Surface enhanced Raman spectroscopy (SERS) is a technique that can be used to enhance Raman signals from molecules to provide chemical information at low concentrations. By coupling the characteristics of a traditional, wide-field microscope with the advantages of SERS, it is possible to image cells on the nanoscale while simultaneously detecting cellular components at a molecular level. For instance, arginine-glycine-aspartic acid (RGD) is a small peptide that binds to receptor proteins responsible significant biological functions in the human body, such as cellular adhesion, signaling, and protein delivery. In this work, surface enhanced Raman imaging was applied via nanoparticle-facilitated introduction of RGD into live cells. Thereafter, localization of RGD-functionalized nanoparticles in cells was determined, and their spectral responses were analyzed to localize the position and chemical interactions of binding to the protein receptors.

(Wed-P14) Analysis of Raman Spectra of Human Primary Keratinocytes and Melanocytes Under Y-Ray Irradiation Exposure

Sila Jin¹, Yeonju Park¹, Hyo-Ji Lee¹, Yu-Jin Jung¹, Young Mee Jung¹; ¹*Kangwon National University*

The various exposure level, physiological condition of the cells, and the dose rate of γ -radiation exposure to human cells influence different biological effects. Biological evaluation by cell viability and changes of cellular organelles can use radiation exposure doses, but it

takes 2-3 days for diagnosis. Therefore, it is necessary to develop biodosimetry techniques and the novel biomarkers identification that can quickly and quantitatively detect radiation exposure doses for clinical application.

Raman spectroscopy is suitable for biodosimetry techniques because it is a non-destructive, non-invasive, quick, and quantitative analysis. Since it provides structural and biochemical information of cells directly, diagnosis of radiation exposure doses is possible by analyzing the specific bands that can change with different radiation doses.

In this study, different radiation doses were exposed to primary human epidermal melanocytes (HEMs) and keratinocytes (HEKs) to investigate damage-related biomarkers capable of detecting γ -radiation exposure doses in the skin. Cell growth, cellular level, intracellular reactive oxygen species (ROS) levels, and changes at the molecular level of HEMs and HEKs before and after γ -radiation exposure were measured using western blot assay, immunofluorescence assay, and Raman spectroscopy. The quantitative detection of the changes of cell membrane composition was also performed using Raman spectroscopy. The detailed results of this study will be discussed in this presentation.

(Wed-P15) **Raman Study on The Toxicity of Amyloid- β to Live Neurons**

Miyu Moriyama¹, Shogo Sato¹, Kosuke Hashimoto¹, Hidetoshi Sato¹; ¹*Kwansei Gakuin University*

The purpose of this study is to elucidate the effects of Amyloid- β (A β) in live neuronal cells using Raman spectroscopy. Amyloid- β is a causative substance of Alzheimer's disease. Symptoms of Alzheimer's disease include neurodegeneration in the cerebral cortex and hippocampus and accumulation of senile plaques. In the recent years, the oligomer hypothesis has been proposed that the oligomers exert toxic effects on neurons. Kawahara et al. reported that the A β oligomers at 10 μ M damaged neurons and caused neuronal death in 24 hours. This report, however, did not describe effect in the functions of the neural cells at lower concentration or with shorter exposure. According to our previous report, Raman spectroscopy was able to detect the molecular changes which had correlation deeply with the functions of live neural cells during their maturation.

The neuronal cells were obtained from hippocampus of rat embryo day 19. The cells were cultured on quartz glass dishes in the incubator (37 °C, 5 % CO₂). The oligomer of A β was prepared from unpolymerized A β in DMSO and PBS. The A β oligomer was added at 10 μ M to the neuron at the day 11 in culturing. Raman spectra of the neurons were recorded at 0 hours, 4 hours, 8 hours, and 24 hours of the exposure to A β . A confocal Raman microscope equipped with a CO₂ incubator is employed for the live cell measurement. The measured spectra were subjected to background removal, baseline correction, and normalization, and then subjected to principal component analysis (PCA) using multivariate analysis software (The Unscrambler).

The results showed that datasets of the A β -treated and untreated cells were categorized into 2 groups in the score plots of PCA, although there was no cell death observed in their dishes. It suggested that the A β oligomer made any reaction with the neurons and was able to disturb their functions. When the datasets with different A β exposure times were compared, the datasets in the PCA score plots were categorized into 3 groups, suggesting

that the reaction of the neurons to the A β oligomer was not acute or severe enough to induce the cell death.

(Wed-P16) Raman Study on Early Reaction in Live Cells Infected with Virus

Momoko Imai¹, Kazuto Takami¹, Keita Iwasaki¹, Kosuke Hashimoto¹, Hidetoshi Sato¹;

¹*Kwansei Gakuin University*

Aim: The purpose of this study is the development of early detection technique of human infectious viruses. Antigen-antibody reaction and PCR tests are the mainstream methods of detecting viral propagation. They are available only to a person who has already had viral infection, because human itself is a filter to select human infectious viruses. Raman spectroscopy can detect changes in molecular composition in live human cells due to viral infection. The previous study suggested that it is possible to detect the viral infection at 3 h, which is an earlier stage than the start of viral DNA replication in the host cell [1]. In the present study, we studied the reaction of the cell infected with a model adenovirus in 2 hours after the infection.

Methods: HEK293 cells were cultured in quartz dishes and infected with a GFP expressing adenovirus. Raman measurements were performed on cell nuclei at 0, 2, 3, and 24 hours after virus infection. HEK293 cells without virus were used as control cells. The Raman spectra obtained from the measurements were background removed, baseline corrected, and normalized. The analysis was performed by principal component analysis (PCA). The GFP expression was observed at 24 hours after infection to confirm adenovirus infection.

Results: The PCA score plots for the datasets with and without the virus measured at 2 h after the infection showed 2 data distributions categorizing the cells with and without virus, although there was a small overlapping. In the loading plot of PCs, the bands attributed to nucleic acids were identified.

Conclusions: The present results demonstrate that the Raman monitoring of live cell is able to identify the viral infection in 2 h at the very early stage after the virus infection.

References [1] Moor, Kamila, et al. "Early detection of virus infection in live human cells using Raman spectroscopy", *J. Biomed. Opt.* 23, 097001-1-7 (2018)

(Wed-P17) Radiation Biodosimetry Using Mouse Hair by Raman Spectroscopy

Spencer A. Witte¹, Courtney J. Morder¹, Zac D. Schultz¹, Naduparambil K. Jacob¹; ¹*The Ohio State University*

Novel methods to detect and quantify radiation exposure without real-time monitoring are important for determining necessary medical treatments, confirming chain of custody of ionizing radiation emitting materials, and verifying exposure levels for environmental monitoring. The effect of ionizing radiation on mouse hair protein structure was studied by acquiring Raman spectra along the shaft of mouse hairs from specimens exposed to varying levels of radiation. Raman spectroscopy is a non-destructive technique that can be paired with optical microscopy to record chemical and spatial information. Hair is a keratotic

biopolymer that usually contains melanin levels sufficient to provide intense luminescence backgrounds over the Raman signal. Therefore, hair samples were subjected to a bleaching treatment before analysis. Changes in the protein structure due to bleaching oxidation were studied by determining the Raman spectra of a virgin, lightly pigmented hair versus the same hair subject to bleaching treatment. Chemometric methods were used to discern differences in control hairs and radiation exposed hairs.

(Wed-P18) Effect of TIR at the air/medium interface on SORS scattering profiles

Kate Whittaker¹; ¹*Agilent Technologies*

We present a Zemax non-sequential mode simulation of Raman scattering, focusing on a system in a spatially offset Raman spectroscopy (SORS) configuration. The simulation is compared to previous results from a monte-carlo simulation [1]. A difference in the depth profile of scattered light that reaches the detector was observed and found to be caused by total internal reflection (TIR) of the scattered light at the interface. We demonstrate that TIR reflects more light into the medium further enhancing signal at depths close to the interface and reducing SORS contrast. We present results that compare how the profile of SORS scattered light varies with varying amounts of reflection and diffusiveness at the air/medium interface.

[1] S. Mosca et al. Analytical Chemistry 2021 93 (17), 6755-6762

(Wed-P19) Classification of Glioblastoma Cancer Stem Cells Using Magnetically Sorted Surfaced Enhanced Raman Spectroscopy and Extracellular Matrix Peptide Mimics

David W. Rist¹, Zac D. Schultz¹, Aleksander Skardal¹, Monica Venere¹, Tom Depalma¹, Miranda Montgomery¹; ¹*The Ohio State University*

Glioblastoma is an extremely heterogeneous and aggressive brain cancer with low survivability which has been found to have cellular subtypes that mimic various stages of brain development. Treating glioblastoma is extremely difficult because within the heterogeneous nature of the cancer, the prevalence of cancer cells with stem cell like properties which self-propagate, and readily adapt to their environment, has led to cancer recurrence in many patients after treatment. The current method for sorting and classifying these cells uses magnetic nanoparticles functionalized with various antibodies such as CD133 and CD 44 to sort via magnetically activated cell sorting and use of RNA sequencing to determine genetic information. This sorting method has been ineffective at improving treatment of these tumors. The tumor microenvironment offers a potential solution to this problem. Fibronectin, laminin, and collagen are all important extracellular matrix (ECM) components that interact with glioblastoma cells through heterodimeric cell surface receptors known as integrins. Functionalizing gold coated magnetic nanoparticles with small peptide mimics for these extracellular matrix components allows for a robust ECM based sort and access to Surface enhanced Raman spectroscopy (SERS). SERS is a

nondestructive ultrasensitive technique which offers a direct method for the identification of these cellular subtypes based on their observed SERS signal. Using SERS, the functionalization of various small peptide ECM mimics for the three main ECM components, such as the well-studied Arg- Gly- Asp (RGD) peptide for fibronectin mimic, can be confirmed and their interaction with their corresponding integrin can be observed allowing for the creation of a library of vibrational spectra that can be used in cell sorting. These peptide-functionalized plasmonic magnetic nanoparticles can then be incubated with glioblastoma cells and serially sorted as to remove the cellular subtypes with a high prevalence of the targeted integrin one at a time to create sorted populations. The sorted cells can then be collected and mapped using SERS with little prep. The resulting maps allow for the classification of different cellular subtypes based on the presence of the integrin signals seen throughout the cell.

(Wed-P20) What Lies Beneath the Surface? – Raman Spectroscopy for Detection of Life in Space

Nicholas Robins¹, Bhavya Sharma¹, Grace Sarabia¹; ¹*University of Tennessee, Knoxville*

Spatially-Offset Raman Spectroscopy (SORS) holds great potential for detection of molecules that indicate life in space. Raman Spectroscopy is a highly sensitive vibrational technique that allows for detection of molecules with high specificity. SORS allows for non-invasive detection through a multilayered sample. On various planets and moons in space, oceans that could have sustained life, are often hidden beneath a thick layer of ice. Development of methods to detect the biosignatures of molecules indicating life beneath ice layers, without having to drill holes in the ice, could be significant for future missions to space. We are developing SORS for detection of these molecules of interest in ice layers, such as those found on Europa. We will demonstrate the viability of SORS for detection of biosignatures in ice-covered oceans through sensing of amino acids, amino acid precursors, and inorganic molecules, previously identified in space as possible biosignatures, through in multi-layered ice samples. [SB1] Based on previous work on SORS in our group, we anticipate detection to depths of > 25 mm.

(Wed-P21) A Wide-Field Imaging Approach for Simultaneous Super-Resolution Surface-Enhanced Raman Scattering Imaging and Spectroscopy

Deben Shoup¹, Zac D. Schultz¹; ¹*The Ohio State University*

The ability to simultaneously obtain high spatial resolution images and chemical specific information is of interest in a variety of biological and physical applications. Surface-enhanced Raman scattering (SERS) is particularly suited for this purpose due to its ability to enhance signal from Raman vibrational modes by probing molecules near the surface of plasmonic metal nanostructures. The spatial resolution in SERS imaging is limited by the diffraction limit of light, limiting the resolution to hundreds of nanometers. However, Raman reporter molecules, such as 4-mercaptobenzoic acid (MBA), adsorbed to single nanoparticles

experience temporal intensity fluctuations that enable the SERS signal to be fit with localization algorithms, such as stochastic optical reconstruction microscopy (STORM). STORM fittings can be applied to generate images with sub-diffraction limited localization of the emitting centers from the nanoparticles. In this work, we demonstrate a wide-field spectrally resolved SERS imaging approach where a transmission diffraction grating placed before the imaging array detector captures the image and first-order diffraction on the same detector. The first-order diffraction corresponds to the SERS spectrum and can be directly correlated to the location and features of a nanoparticle. We show that spatially correlated Raman spectra from multiple MBA-functionalized nanoparticles in a wide-field of view are readily obtained on a 10-100 ms time scale. This enables spatially resolved monitoring of chemical processes, such as intermediate formation resulting in frequency fluctuations in the observed spectra, which are thought to arise from the transient capture of energetic charge carriers from the nanoparticles to MBA.

(Wed-P22) Raman Spectroscopic Determination of Cellular Composition in Novel 3D Neuronal Cell Cultures

Natalie Dunn¹, Meaghan Harley¹, Emily Travis¹, Wilson A. Garuba¹, Avery Wood¹, Larry Millet¹, Madhu Dhar¹; ¹*University of Tennessee, Knoxville*

Neurological diseases are highly complex and challenging to diagnose and treat. Most neurological diseases, particularly neurodegenerative diseases, are diagnosed based on the presentation of symptoms, when the disease is already established, and there are little to no methods developed for early disease detection. We are pursuing the development of Raman spectroscopy-based techniques for early detection of neurological disease. As part of our focus on neurological conditions, we are interested in probing cells and neurochemicals at the cellular level with Raman spectroscopy. Neurological disease research and treatment often relieve neural stem cells, which are highly sensitive cells and difficult to culture. We have recently developed methods for 3-dimensional neuronal cell culture derived from primary cells, where cells are isolated from rodent hippocampal tissue and proliferated. The 3D neurospheres are robust and differentiate more easily than stem cells. We will present results on the characterization and viability of the 3D neurospheres with standard methods, such as fluorescence imaging, and with Raman spectroscopy. We will demonstrate the promise of applying Raman spectroscopy towards differentiating between the components of the neurospheres, including neurons and glial cells, in living (not fixed) cells. We also will present results on Raman spectroscopic characterization of brain tissue.

(Wed-P24) Detection and Monitoring of Neuroinflammation With Surface Enhanced Raman Spectroscopy.

Wilson A. Garuba¹; ¹*University of Tennessee, Knoxville*

In 2016, Global Burden of Diseases (GBD) stated that neurodegenerative disorders (NDs) were the leading cause of disability-adjusted life-years (DALYs) and the second leading cause of death worldwide. NDs, including Parkinson's and Alzheimer's diseases, are

prevalent in elderly individuals. As life expectancy has been forecasted to be significantly extended around the world due to technological and scientific advancement, the physical, emotional, and financial burdens of NDs are expected to mount. Thus, there is an urgent need for understanding the mechanisms driving neurodegenerative diseases to develop improved treatment techniques. Neuroinflammation is intimately related to NDs; this has sparked significant interest in the study of inflammatory response in the brain. We seek to address neuroinflammatory responses in the brain and the resulting neurodegenerative diseases using Raman spectroscopy. Raman spectroscopy is non-destructive, rapid, utilizes label-free samples, and provide high specificity with unique chemical signatures, making it spectroscopy superior to other techniques used in neuroinflammation studies. Raman scattering, however, is an inherently weak scattering process. For increased signal enhancement, we combine a property of noble metal (Au and Ag) nanoparticles (NPs) known as the localized surface plasmon resonance (LSPR), with Raman scattering to yield intensity enhancement up to nine orders of magnitude, known as surface enhanced Raman spectroscopy (SERS). The NPs can be synthesized in various shapes and sizes, making them valuable SERS substrates due to their malleability. We will present results demonstrating development of SERS substrates to probe neuroinflammation. We aim to provide an understanding of neuroinflammatory responses in the brain and aid in developing sensors for early disease detection and potential treatment methods.

(Wed-P26) Raman Spectroscopy and chemometrics: A Potential Method for Fingerprint Discrimination from Gentle Touch of Drugs Tablets.

Mohamed O. Amin¹, Entesar Alhetlani¹, Igor K. Lednev²; ¹*Kuwait University*, ²*University at Albany, State University of New York*

Recent advancements in analytical techniques have greatly contributed to the chemical analysis of FMs and identification of materials that a suspect might have ingested or come into contact with. This type of information about the FM donor is valuable for criminal investigations because it narrows the pool of suspects and offers information about the individual's habits. It is estimated that at least 30 million people around the world take over-the-counter (OTC) and prescription nonsteroidal anti-inflammatory drugs (NSAIDs) for pain relief every day. The present study demonstrates the potential of Raman spectroscopy combined with multivariate statistical analysis as a rapid and nondestructive technique for the detection and identification of drug traces in LFMs when an NSAID tablet has been touched. Specifically, aspirin, ibuprofen, diclofenac, ketoprofen and naproxen tablets purchased from a local drugstore were used to produce NSAID-contaminated FMs. Despite the presence of similar functional groups in these drugs, subtle differences were observed in their Raman spectra. Partial least squares discriminant analysis (PLS-DA) showed an excellent separation between natural FMs and all NSAID-contaminated FMs. The developed classification model was externally validated using FMs deposited by a new donor and showed 100% accuracy on a FM level. This proof-of-concept study demonstrated the great potential of Raman spectroscopy in the chemical analysis of LFMs and the detection and identification of drug traces in particular.

(Wed-P27) Effect of Hormone Replacement Therapy on Sex Determination Through Raman Spectroscopy

Emily Miller¹, Brooke W. Kammrath¹, Igor K. Lednev², Alexis R. Weber²; ¹*University of New Haven*, ²*University at Albany, State University of New York*

Hormone replacement therapy (HRT) is a common treatment for a variety of individuals, including women taking estrogens or progestogens to alleviate the symptoms of menopause, men taking testosterone to combat the natural decrease in its production with aging, and transgender or nonconforming individuals taking hormones to more closely align their secondary sexual characteristics with their gender identity. In the case of transgender hormone therapy, there are two types: masculinizing hormone therapy which provides androgens and antiestrogens to transgender men or transmasculine people, and feminizing hormone therapy in which estrogens and antiandrogens are given to transgender women or transfeminine people.

Over the last 15 years, meaningful research has been published on the use of Raman spectroscopy for the determination or classification of sex from blood and dried blood deposits. It is known that there are differences in the makeup of blood between females and males such as variations in the coagulation factor FV, α 1-antitrypsin, and β 2-macroglobulin found in plasma. Thus, the ability of Raman spectroscopy combined with chemometrics to differentiate blood samples by the biological sex of the donor is scientifically seen.

However, given that HRT introduces exogenous hormones which will affect a person's biochemistry, especially in cases of transgender HRT, it is important to investigate the effects of HRT on the classification of sex by Raman spectroscopy. This research investigated how chemometric models that have been used for sex classification work when tested with samples from transgender individuals undergoing HRT. Transgender and nonconforming individuals are disproportionately victims of violent crimes; however, they are not often included in scientific research, including forensic science research. It is essential to include this underrepresented group within the LGBTQ+ community to ensure they are represented in all forensic science, especially in research such as this where analytical chemistry is used to determine the sex of the donor of a blood deposit.

(Wed-P28) Chemical Effects in Protein Analysis: A Systematic Investigation of Amino Acid Spontaneous Raman and SERS Responses

Richard A. Dummitt¹, Zac D. Schultz¹; ¹*The Ohio State University*

Spontaneous Raman spectroscopy and surface enhanced Raman spectroscopy (SERS) are powerful and non-destructive tools for detection and characterization of biomolecules such as proteins, peptides, and the amino acids that comprise them. SERS is viewed as having more practical applications as opposed to spontaneous Raman due to its magnification of the inherently weak Raman scattering signal, allowing for improved detection of analytes at low concentrations. However, spectral differences have been observed within literature between spontaneous Raman and SERS spectra of amino acids such as tryptophan that have led to issues in effectively analyzing protein and peptide structures via this method. The cause of these spectral differences is not yet well understood; however, it is believed they

are a result of chemical mechanisms such as charge transfer effects and chemical conversion. In some cases these mechanisms appear to selectively enhance specific amino acids. It is thought that chemical features such as aromatic rings and side-chain nitrogen found within amino acids such as tryptophan and arginine respectively contribute to greater occurrence of these enhancing effects, and therefore greater magnification of Raman signal by SERS relative to other amino acids. In this work, each amino acid's limit of detection is calculated and compared between spontaneous Raman and SERS. Using these observed changes in limit of detection across each amino acid, two separate amino acids can be compared to determine if similar chemical features such as aromatic rings are leading to appreciable differences in SERS enhancement compared to those amino acids lacking these features. By better understanding the relationship leading to these differences in SERS enhancement, the chemical mechanisms behind the cause of SERS spectral differences within amino acids may be better understood, leading to more effective application of SERS for analysis of not just amino acids and proteins, but application of SERS as an analytical tool.

(Wed-P29) Liquid Chromatography - Sheath Flow Surface Enhanced Raman Spectroscopy for Identification of Resveratrol in Red Wine

Kristen Wang¹, Zac D. Schultz¹; ¹*The Ohio State University*

Resveratrol is a stilbenol molecule found in red wine that may have many health benefiting properties, such as preventing inflammation in blood vessels. This metabolite is found naturally in grapes and is one of the reasons why red wine is promoted as healthy enough to drink a glass everyday before bed. This project aimed to quantify the amount of resveratrol in a sample of red wine using LC-SERS. SERS is a non-destructive quantitative method that measures the inelastic scattering of light, where the signal is enhanced by adsorbing the sample on a metal nanostructure. For this project, a thermally evaporated silver substrate placed in a homemade flow cell for SERS detection. Red wine was separated through by using reverse phase chromatography before flowing into the flow cell where the SERS signal was obtained. A calibration curve of resveratrol was made by flowing different concentrations of resveratrol through the flow cell. Once that was achieved, the resveratrol was injected into the HPLC to determine the retention time. By separating resveratrol from the other components of red wine, the SERS signal can be compared to the reference spectrum for identification and intensity provides quantitative information. Data analysis took place on MATLAB, using airPLS as a background subtraction method and Pearson correlation to compare sample spectra with a reference spectrum.

(Wed-P30) “Point of use” And Non-destructive Qualitative Screening of Long-lasting Insecticidal Mosquito Nets With Handheld Raman Spectroscopy For Malarial Prevention

Ed Bethea¹, Matt Eady¹, David Jenkins¹; ¹*FHI360*

Traditional testing of long-lasting insecticidal mosquito nets (LLIN)s for Malarial prevention requires destructive and time-consuming sampling which involves cutting

sections from various locations in the net. This approach to net screening for quality compliance is time consuming, costly, and does not allow for a large quantity of samples to be analyzed in a short amount of time. Raman spectroscopy provides detailed information about chemical structure based on the interaction of light with the chemical bonds in a material. Here, a portable handheld Raman spectrometer equipped with a 1064 nm laser ($141 - 2495 \text{ cm}^{-1}$) is used to qualitatively classify the net material type (polyester and polyethylene) and insecticide (alpha-cypermethrin, deltamethrin, permethrin, and blank nets) present through rapid and non-destructive methods. LLINs were collected from 12 manufacturers over the span of 37 production months ($N = 291$). Swatches were packed into glass vials and scanned with the handheld Raman spectrometer. Discriminate analysis (DA) was used to predict the net material type results from the collected spectra of each sample. The Raman spectrometer found that qualitative differentiation of LLIN material type and insecticide performed at 100% accuracy. The portable handheld, “point of use”, Raman spectrometer shows promise in its ability to qualitatively screen LLIN swatches without cutting the net or costly and time-consuming quality compliance methods.

Novelty statement: Material type and low levels of insecticide types could be differentiated on mosquito nets for Malarial prevention using portable Raman spectroscopy.

(Wed-P33) Raman Spectroscopy: An Effective Analysis Tool for Lithium-ion Battery Manufacturing and Quality Control Processes

Bruno Beccard¹, Shaileshkumar Karavadra¹, Sudhir Dahal¹; ¹*Thermo Fisher Scientific*

The efficiency of renewable energy technologies such as solar and wind power have drastically increased over the last decade. In addition, the cost of owning these technologies by households as well as energy companies has gone down significantly. This has resulted in a huge demand for energy storage devices, including batteries. Of particular interest is the development and improvement of portable lithium-ion (Li-ion) batteries because of their advantages over traditional battery technologies. The most notable advantage of Li-ion batteries includes highest energy density compared to other existing rechargeable batteries making them faster to charge and long-lasting even in high-demand needs such as powering electric cars, powering electric machineries and as energy source for households.

Li-ion batteries continue to get better; however, since the technology is still evolving, it is far from perfect. There have been several high-profile reports of failures, including explosion and fire caused by Li-ion batteries during charging, use and storage. Therefore, a variety of research is underway to make the technology more efficient, useful for a variety of applications, and safe.

Here, we present Raman spectroscopy (including Raman imaging) as a suitable technique of choice for characterization and analysis of Li-ion batteries during the manufacturing process. We highlight two cases of bulk analysis of lithium compounds using Raman spectroscopy during the quality control procedure of raw materials, and one case of analysis for better manufacturing using Raman imaging microscopy.

Wednesday Poster Session - SPECIAL

(Wed-P34) Dimethyl Carbonate as a Mobile Phase Modifier for Normal Phase and Hydrophilic Interaction Liquid Chromatography

Philip Boes¹, Sophie Elleman¹, Neil Danielson¹; ¹*Miami University*

We have found dimethyl carbonate (DMC) to be a versatile mobile phase modifier for liquid chromatography (LC). DMC is a moderately polar modifier with a low UV-cutoff for normal-phase LC. A comparison of ethyl acetate (EA) and DMC as organic mobile phase modifiers in hexane for normal phase LC of phthalates, common plasticizers with environmental and health concerns, using a core-shell silica column was conducted. Data were collected through the separation of dioctyl phthalate, dibutyl phthalate, benzyl butyl phthalate, diethyl phthalate and dimethyl phthalate on a silica column in varying composition of hexanes with EA or DMC (from 90 to 98% hexane and 10 to 2% modifier). Retention factors (k) ranged from < 1 to 5 with EA and around 1 to 10 with DMC while plate counts remain similar. Detection at 215 nm, possible with DMC, allowed for better detection of the phthalates by a factor of 10, compared with EA detection, best at 254 nm. We have also shown DMC, being aprotic, can be an effective non-toxic mobile phase substitute for acetonitrile (MeCN) for hydrophilic liquid interaction chromatography (HILIC). The low solubility of DMC (~14% v/v) with water requires the aqueous part of mobile phase to have one part of ethanol mixed with two parts of ammonium acetate buffer. Using a core-shell silica column, separations of trans-ferulic acid, syringic acid, and vanillic acid were compared between MeCN and DMC as the organic portion of the mobile phase at the percentage range from 85 – 95%. The analyte retention for DMC when compared to MeCN was about 1.5 times greater with only a moderate increase in back pressure. Plate count and peak asymmetry were comparable for both DMC and MeCN mobile phases. Seven mono- and di-hydroxybenzoic acid positional isomers could be resolved effectively with DMC. Sorbate and benzoate preservatives in commercial drinks were also determined.

(Wed-P35) Applications of Digital Microscopy for the Analytical Chemistry Teaching Laboratory

Hannah Newell¹, Krista Wilson¹, Alexander Igwebuiké¹, Andre J. Sommer¹, Neil Danielson¹; ¹*Miami University*

In our upper level analytical chemistry class, digital microscopy has been applied to teaching analytical chemistry in two ways as an end-of-semester class project. First, the qualitative analysis of various transition metal ion at the ppm (mg/L) level using precipitation chemistry and digital microscopy has been done. Precipitates are generated by mixing two microliter drops of the metal and precipitating reagent on a Teflon-tape covered slide. Precipitate color detection is made using a captured image of the pre-rinsed dried precipitate by digital microscopy. Many transition metals are considered toxic so doing these reactions with microliter droplets of low ppm concentrations is much safer and reduces waste. Precipitate formation for nickel using dimethylglyoxime, cobalt using 2-nitroso-1-naphthol, and lead using rhodizonate as a function of concentration was possible at the 10 ppm level. Secondly, a fluorescence microscope was built using a 365 nm black

light pen flashlight excitation source mounted close to the digital microscope. Determination of ppm levels of riboflavin in microliter droplets permitting its estimation in a supplement was facile. Fluorescent metal complexes of Mg, Ca, and Zn were generated using 8-hydroxyquinoline-4-sulfonic acid (HQSA) for detection at the ppm level and various supplements containing each of those metals were also tested. The Mg-HQSA complex provided strong fluorescence at 10 ppm. This instrument clearly demonstrated the possibility of microdroplet instead of cuvette based fluorescence spectroscopy. Digital microscopes are easily portable and quite inexpensive, allowing for all images to be easily saved on a flash drive for later analysis. Quantitative determination of the spot intensity was possible with qTLC open source software.

Wednesday Poster Session - SPR

(Wed-P36) Comparing Localized Surface Plasmon Resonance on Single Gold Sphere Nanoparticle and Nanorod Using Two-Trace Two-Dimensional Correlation Spectroscopy

Sila Jin¹, Yeonju Park¹, Young Mee Jung¹; ¹*Kangwon National University*

The localized surface plasmon resonance (LSPR) observed in nanoparticle has different characteristics according to shape, size, metal species, structure, and so on. Since surface-enhanced Raman scattering (SERS) effect depends on LSPR, SERS spectrum of even the same target can be changed with condition of nanoparticles. There are a lot of fundamental and application studies on LSPR in various fields. Although there are investigations of correlation between LSPR and SERS spectra, a detailed understanding of which bands in the SERS spectra correspond to LSPR are still lacking.

Two-dimensional correlation spectroscopy (2D-COS) is a very powerful spectral analysis method. It provides much more information than conventional spectral analysis; elucidation of inter- or intra-molecular interaction, enhancement spectral resolution, and determination of spectral intensity changes during external perturbation. Among the 2D-COS, two-trace two-dimensional correlation spectroscopy (2T2D) can provide such information using only two spectral data.

In this study, to elucidate the correlation LSPR and SERS spectra, 2T2D was applied to the SERS spectra of the SERS probe absorbed on the single gold sphere nanoparticle and nanorod. The details of the results will be discussed in this presentation.

(Wed-P37) Construction of Solid-State Plasmonic Rulers Comprising Sharp Tip Gold Nanostructures tethered with Photoswitchable Molecular Machines

Sarah R. Langlais¹, Sumon Hati¹, Rajesh Sardar¹; ¹*Indiana University - Purdue University Indianapolis*

Plasmonic rulers link nanoscale distance changes to observable spectral shifts that are very important for the study of cellular microenvironments and the detection of both single and low abundant biomarkers. Traditional plasmonic ruler design employs the plasmonic coupling between adjacent nanoparticles that have historically been hindered by

reproducibility issues, specifically controlling distances. To overcome this challenge, herein we report the fabrication of a novel plasmonic ruler containing chemically synthesized sharp tip gold nanostructures tethered with photoswitchable molecular machines linked via alkylthiolate chains. This unique and highly sensitive plasmonic ruler utilizes localized surface plasmon resonance (LSPR) properties of gold nanostructures to spectroscopically evaluate plasmon-dipole coupling between nanostructures and photoisomerizable spiropyran (SP)-merocyanine (MC) conjugates in the solid-state. We studied three different shaped gold nanostructures, i.e. triangular nanoprisms, nanorods, and nanobipyramids, to determine which shape provides the maximum distance dependent LSPR response. The resulting plasmonic rulers were characterized both spectroscopically, and with density functional theory (DFT) and finite-difference time-domain (FDTD) calculations to elucidate the plasmon-dipole interaction as a possible mechanism. The novel and highly innovative plasmonic ruler fabricated in this work represents a new class of plasmonic rulers that are no longer restricted to nanoparticle dimer constructs or impeded by reproducibility issues. In addition, this next generation plasmonic ruler holds great promise for advanced, plasmonic-based sensor and optoelectronic device fabrication.

(Wed-P38) Investigation of Electronic Interactions Influencing the Plasmonic Property of Conjugated Ligand-Passivated Gold Nanostructures

Sumon Hati¹, Xuehui Yang², Jing Zhang², Rajesh Sardar¹; ¹*Indiana University - Purdue University Indianapolis*, ²*Indiana University Purdue University Indianapolis*

Surface modification of plasmonic nanostructures is fundamentally important to enhance optoelectronic properties for advanced applications. In this context, the electronic structure of surface passivating ligands at the metal-organic hybrid interface can modulate the plasmonic response of noble metal nanostructures. However, there is still a gap in knowledge in understanding the physical and chemical processes that occur at the metal-organic hybrid interface. Here, we present a new metal-organic hybrid system in which gold triangular nanoprisms (Au TNPs) were chemically modified with conjugated cinnamic acids ligands using thiolate as an anchoring group to form a covalent Au-S bond. Altering the substitution at different positions of the aromatic group ranging from electron withdrawing (-CF₃, -NO₂) to electron donating (-CH₃, -OCH₃, -N(CH₃)₂) in comparison to a hydrogen substitution (-H) provided ~80 nm reversible shifts of localized surface plasmon resonance (LSPR) dipole peak of TNPs in solid-state. Furthermore, ultraviolet photoelectron spectroscopy characterizations showed a large change in Au work function, which is determined to be a function of ligands' dipole moment. We conducted the density functional theory (DFT) calculations to examine the role of ligand's dipole moment (induced and permanent dipole moment) in the metal-organic hybrid system altering the permittivity and electron transfer across the Au-S interface. Moreover, our density of states (DOS) calculation shows changes in the electronic structures near the Fermi level further supporting the experimental observation. In-depth spectroscopic study shows that the pi-conjugation in the thiocinnamic acid allows delocalization of transferred electron into aromatic backbone that results in an addition of red-shift in the dipole peak position of TNPs. Together, our combined experimental and theoretical studies delineating the

fundamental mechanistic understanding of uniquely designed ligand-passivated plasmonic nanostructures should expedite the potential applications in various fields such as energy conversion and storage, and sensing.

(Wed-P39) Exploring Optimal Gold Nanoparticles for Single Particle Surface-Enhanced Raman Scattering Sensing

Sanjun Fan¹, Brian Scarpitti¹, Zac D. Schultz¹; ¹*The Ohio State University*

Surface enhanced Raman scattering (SERS) is a powerful analytical technique that offers non-invasive, rapid, multiplex, and highly sensitive detection of chemical analytes in a wide variety of applications including material science and biochemical sensing. The SERS signals can be significantly enhanced by several orders of magnitude (10^{8-15}) to enable optical detection of single molecules through the amplification of electromagnetic fields associated with light interacting with nanostructured materials such as Au, Ag, and Cu. Studies in engineering plasmonic nanoparticles have shown the field enhancement is very unevenly distributed and mainly located on “hot spots”, often in the form of very sharp tips on nanoparticles or nanogaps between nanoparticles. To understand the SERS signals generated by different plasmonic nanostructures and the environmental impacts to them, it is valuable to investigate single particle images and spectra that are often hidden in ensemble-averaged measurements. However, it is still challenging to detect SERS signals at single particles level in solution and this could be ascribed to 1) instability of signal due to the orientation of asymmetrical nanoparticles in solution; and 2) very low SERS signal at single particle level that can be transiently observed in solution. Herein, we have synthesized dozens of gold or silver nanoparticles with various morphologies including symmetric and asymmetric nanostars, nanorods, nanobipyramids, nanospheres, core-shell Au/Ag, Au-Ag alloy nanostars, gap-enhanced Raman tags (GERTs), etc, and then compared the SERS effects observed from Raman labels attached. We found that the GERTs nanoparticle are optimal choice for single particle SERS because these particles not only exhibit superior SERS signals, but also show increased relative stability among these nanoparticles. Addressing these challenges will increase the utility of SERS at single particle level for our future projects in the quantification nanoparticles in cells and bioanalytical sensors.

(Wed-P40) Effects of Nanoparticle Multiplicative Scattering on Optical Spectroscopic Measurements

Samadhi N. Nawalage¹, Pathum D. Wathudura¹, Dongmao Zhang¹; ¹*Mississippi State University*

The effects of light scattering on the experiment quantification of materials absorption, scattering, and emission properties in turbid solutions have been controversial in literatures. Herein we present a computational and experimental studies on the effects of nanoparticle multiplicative scattering on the experimental quantification of nanoparticle scattering intensity and depolarization. Multiplicative light scattering introduces both

scattering depolarization and sample inner filter effect (IFE). The latter refers to the nonlinearity between the scattering intensity and nanoparticle concentration, as well as the distortion of the scattering intensity spectra. While the degree of the IFE caused by light scattering is significantly smaller than that by the sample light absorption, correcting scattering IFE is challenging because such IFE is strongly polarization dependent. Another notable finding is that light scattering depolarization is more robust than scattering intensity for nanoparticle quantification. The insights provided in this work is important not only for using light scattering as a materials characterization tool including nanoparticle quantifications, it is also critical for quantitative mechanistic understanding on the effects of light scattering on sample absorption and fluorescence measurements.

Thursday, October 6, 2022

Oral Presentations

22AWD10: FACSS Innovation Award Finalists Plenary Session

Chair: Karen Faulds

(AWD-10.3) Biomimetic Transparent Nanoplasmonic Meshes by Reverse-Nanoimprinting for Bio-interfaced Spatiotemporal Multimodal Surface-enhanced Raman Spectroscopy

Aditya Garg¹, Elieser Mejia¹, Wonil Nam², Peter J. Vikesland¹, Wei Zhou¹; ¹*Virginia Tech*, ²*Virginia Tech*

Biomimetic nanoplasmonic meshes for spatiotemporal SERS analysis of living biosystems in targeted and non-targeted modalities.

Multicellular systems, such as microbial biofilms and cancerous tumors, feature complex biological activities coordinated by cellular interactions mediated via different signaling and regulatory pathways, which are intrinsically heterogeneous, dynamic, and adaptive. However, due to their invasiveness or their inability to interface with native cellular networks, standard bioanalysis methods do not allow in situ spatiotemporal biochemical monitoring of multicellular systems to capture holistic spatiotemporal pictures of systems-level biology. Here, we report a high-throughput reverse nanoimprint lithography approach to create biomimetic transparent nanoplasmonic microporous mesh (BTNMM) devices with ultrathin flexible microporous structures for spatiotemporal multimodal surface-enhanced Raman spectroscopy (SERS) measurements at the bio-interface. We demonstrate that the BTNMMs, carrying dense and uniformly-distributed nanolaminated plasmonic nanoantenna (NLPNA) arrays that support multiple hybrid localized surface plasmon (LSP) modes with spatial mode overlap, can serve as highly sensitive SERS devices (SERS enhancement factor $> 10^7$) for spatiotemporal multimodal SERS measurements in both targeted and non-targeted modalities. As a proof of concept demonstration, we show that the BTNMMs can simultaneously enable spatiotemporal SERS measurements for targeted pH sensing and non-targeted molecular detection to resolve the diffusion dynamics for pH, adenine, and Rhodamine 6G molecules in agarose gel. Moreover, the BTNMMs are employed as multifunctional bio-interfaced mesh SERS sensors to conduct in-situ spatiotemporal pH mapping and molecular profiling of *E. coli* biofilms. We envision that the biomechanical

compatibility, transport permeability, and ultra-sensitive multimodal SERS capability of the BTMNNs can open exciting avenues for bio-interfaced multifunctional sensing applications both in vitro and in vivo.

(AWD-10.4) Rapid Vibrational Circular Dichroism – Opportunities through the combination of External Cavity Quantum Cascade lasers and balanced detection

Daniel-Ralph Hermann¹, Georg Ramer¹, Bernhard Lendl¹; ¹*TU Wien*

Maximizing the advantages of tunable Quantum Cascade lasers for low noise Vibrational Circular Dichroism

Chirality is an important aspect of the chemical and biological world, being present prominently in our own body. Consequently, it has implications for the pharmaceutical industry, as the absolute configuration of an analyte influences its therapeutical index. Due to this fact, Vibrational Circular Dichroism (VCD), which can assess the chirality of an analyte in solution, has become a routinely used tool when dealing with active pharmaceutical ingredients. However, while VCD, which operates in the IR spectral region, is broadly applicable, it suffers from a signal intensity about 10^5 times weaker than classical IR absorbance spectroscopy. This circumstance necessitates long measurements (several hours) to collect reliable VCD spectra.

Quantum Cascade Lasers (QCL) are tunable mid-IR lasers, offering intense and highly polarized emission in the IR region. For classical IR spectroscopy, QCLs already outperformed classical FT-IR in terms of limits of detection and signal-to-noise ratios (SNR) even in demanding aqueous solvent systems. QCL based VCD instruments are also able to leverage the inherently linearly polarized light as it eliminates the need for a polarizer. However, QCLs come with disadvantages not present in thermal light sources, mainly thermal drifts and pulse to pulse intensity fluctuations. These phenomena introduce noise into the measurement system, possibly offsetting the advantages gained from employing a QCL.

To counter these drawbacks, we combined a QCL based VCD setup with a balanced detection system. In balanced detection, the laser beam is split into two beams, which are directed onto two detectors. The detector signals are then subtracted. Laser noise, present in both channels, is compensated, while the analyte information, only present in one channel, is still accessible. After adapting this scheme to VCD, we reported a noise level decreased by a factor of 4 compared to single detector systems. Compared to classical FT-IR, we report a decrease in noise by a factor of at least 2 within a third of the measurement time. With this increase in measurement speed, QCL based VCD has the potential to be used as monitoring tool for more dynamic process, like chiral reactions or protein folding.

(AWD-10.1) What Lies Beneath your Elution Peak: Imaging When and Where Analytes Adsorb to Commercial Stationary Phase Particles

Lydia Kisley¹, Lydia Kisley¹, Ricardo Monge Neria; ¹*Case Western Reserve University*

Studying separations from the bottom-up — one molecule at a time — can identify rare events that lead to the failure of challenging, high-purity separations that are hidden in conventional experiments (chromatography, isotherms, breakthrough curves, *etc.*). Single-molecule fluorescence microscopy, an optical imaging technique, resolves spatiotemporal nanoscale dynamics that occur in separations. Millisecond time resolutions and sub-diffraction limited, 3D spatial resolutions at ~10 nm are achieved *in situ*. Single-molecule fluorescence microscopy has previously detected and quantified the causes of common problems in reverse-phase, normal-phase, ion-exchange, and membrane separations, yet prior sample conditions were obtained from overly-simplified model substrates and low concentrations of a single analyte. Here, we expand single molecule microscopy to quantifying analyte dynamics on commercial chiral stationary phase particles. We map the adsorption sites in 3D over at ~10 nm resolutions showing rhodamine 6g analytes only adsorb to the edges but do not enter the interior of the particle of fully porous particles. We quantify adsorption rates, desorption rates, and free energy at individual binding sites, showing intra- and interparticle heterogeneity. Our results demonstrate how single molecule microscopy can reveal the underlying adsorption-desorption phenomena that lead to peak broadening in bulk scale separations.

(AWD-10.2) **Opto-Lipidomics of Tissues**

Mads S. Bergholt¹; ¹*King's College London*

We demonstrate the first integrated Raman and mass spectrometry instrument paving for (optical) lipidomics

Here we drive forward a new paradigm of Raman technology that enables optical lipidomic analysis of tissues. Raman spectroscopy is a label-free optical technique that can provide a point-wise vibrational molecular fingerprint of tissue “optical biopsy” for tissue diagnosis. State-of-the-art Raman spectroscopy, however, does not offer specific compositional analysis or insights into molecular biology of tissue hindering widespread adoption. This is because the vibrational Raman bands are overlapping and cannot be deciphered into the myriad of biomolecules in complex tissue. We introduce a new instrument and methodology to enable Raman lipidomic analysis. To this end, we developed a novel integrated Raman and mass spectrometry imaging system for pixel-wise correlation. We demonstrate that multivariate regression can be used to translate from the vibrational structural domain (Raman spectroscopy) into the more specific compositional lipidomic domain (mass spectrometry) thereby enabling optical lipidomics.

22PLEN04: AES Electrophoresis Mid-Career Award

(PLEN-04.3) **Nonlinear Electrophoresis of Colloidal Particles**

Aditya Khair¹; ¹*Carnegie Mellon University*

The past decade has witnessed a surge of interest in nonlinear electrophoresis of charged colloidal particles in aqueous electrolytes. Here, the word nonlinear refers to the fact that the ratio of the electrophoretic speed of the particle to the magnitude of the applied electric field—the electrophoretic mobility—is not independent of field strength. This is in stark contrast to the vast majority of work on (linear) colloidal electrophoresis over the last century, where the mobility is assumed to be a material property dependent only on the particle–electrolyte combination. In this talk, I will first review various experimental measurements of the field-dependent mobility. I will then discuss theoretical approaches to predicting the nonlinear mobility, including asymptotic schemes in the common thin-Debye-layer limit and our own recent computations via direct numerical simulation of the full electrokinetic equations. I will conclude with suggestions for future work and opportunities in this evolving area of electrophoresis.

22PLEN04: ANACHEM Award

(PLEN-04.2) Mass Spectrometry Au Naturel: A Tool for Structural Biology

Joseph A. Loo¹; ¹*University of California, Los Angeles*

Native mass spectrometry (MS) of proteins and protein assemblies reveals size and stoichiometry. But elucidating their structures to understand their function is more challenging. What “structures” are really being probed by native MS? As defined by Leney and Heck (*J. Am. Soc. Mass Spectrom.* 2017), the term “native” in “native MS” refers only to the biological status of the protein/biological assembly in solution prior to mass analysis. But to what extent are native protein structures preserved in the gas-phase (and are being measured by MS)? We show that native MS and native top-down MS can be effective for deriving structural information for soluble and membrane protein complexes, and much of this information can be correlated to the solution-phase structure. Native top-down MS generates information on the surface topology, ligand binding sites, and post-translational modifications (PTMs) of protein complexes. We use native MS/MS to investigate the molecular action of compounds that prevent amyloid fibril formation in neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease. Electrospray ionization’s gift for transforming solution-phase macromolecules into gas-phase ionized counterparts without disrupting weak non-covalent interactions is key for applying MS to study protein complexes. But questions remain, including whether the measurement of gas-phase protein ions relate to the structure of proteins in solution? And as posed nearly 25-years ago (Loo, *Mass Spectrom. Rev.* 1997), “Is there any utility in studying the structure of the gas-phase protein complex?”

22PLEN04: SAS and Applied Spectroscopy William F. Meggers Award

(PLEN-04.1) Process Analytical Utility of Raman Microspectroscopy for Cell Therapy Manufacturing Validation

James Piret¹, Robin Turner¹, Georg Schulze¹, Shreyas Rangan¹, Martha Vardaki², Diepiriye Iworima¹, Timothy Kieffer¹, Michael Blades¹; ¹*The University of British Columbia*,
²*Institute for Chemical Biology*

New clinical therapies based on implanting living cells into patients offer great promise to cure degenerative and deadly diseases, including Type I diabetes and cancers, with hundreds of clinical trials initiated yearly. This huge potential comes with major challenges, including that populations of living cells are far more complex (and inherently variable) than any molecular therapeutic. Furthermore, they cannot be either purified or analyzed anywhere near as stringently. The long-term success of cell-based therapeutics will depend largely on the development of improved methods to validate both the final cell product quality as well as normal critical process parameter levels during the manufacturing processes. Raman spectroscopy offers a non-invasive and label-free approach to distinguish cell types and physiological states by analyzing cellular macromolecular composition changes. Our group is working towards developing process analytical technologies for several cell therapies based on Raman spectroscopy. This plenary lecture will focus on using Raman microspectroscopy to follow a directed differentiation of stem cells to yield insulin-producing pancreatic cells for treating Type I diabetes, a manufacturing process that involves seven stages of differentiation. We have shown that spectral markers, such as the ratio of nucleic acid to protein-associated band intensities, can discriminate between stem cells and their differentiated progeny. Other spectral markers distinguish off-target cell type emergence, and the measurement of disulfide bond related features correlate strongly with increasing insulin levels in on-target differentiating cells. Thereby these measurements can be used to infer both the purity and potency of the manufactured cells. Raman spectroscopy thus offers a promising approach to provide a highly informative, non-destructive means to validate the quality of cell therapy manufacturing.

22ATOM03: Nuclear

Chair: Benjamin Manard

(ATOM-03.1) Direct Analysis of Swipe Surfaces for Uranium by a Novel Microextraction-ICP-MS Approach

Benjamin T. Manard¹, Brian Ticknor¹, Veronica Bradley¹, Cole R. Hexel¹, Shalina Metzger¹, Tyler Spano¹; ¹*Oak Ridge National Laboratory*

Traditional nuclear safeguards analysis will routinely utilize environmental swipes to survey various locations within a nuclear facility. These swipes can then be analyzed in entirety (i.e. bulk analysis) such that any information regarding its actinide constituents can be determined. This information can provide insight regarding the nuclear materials or enrichment process that may be present within the facility. The techniques to analyze these environmental swipes are rather laborious, including ashing, dissolution, and separations prior to high precision analysis by multi collector-inductively coupled plasma-mass spectrometry (MC-ICP-MS). The work presented here investigates an approach to directly extract the analytes from the environmental swipe surface and determine isotopic abundances, via inline ICP-MS detection. This methodology, microextraction-ICP-MS, is employed such that a microextraction probe head lowers onto the environmental swipe surface, seals on the sample surface, and delivers an extraction solvent (i.e. 2% HNO₃) to subsequently desorb target analytes from the surface. The flowing solvent, with extracted analyte, is directed into the ICP-MS for actinide isotopic determination. Initial evaluation of the microextraction-ICP-MS methodology was successful at determining the isotope

ratios ($^{234}\text{U}/^{238}\text{U}$, $^{235}\text{U}/^{238}\text{U}$, and $^{236}\text{U}/^{238}\text{U}$) in a series of certified reference materials (CRMs) deposited onto swipe surfaces.

(ATOM-03.2) Predicting Gas Phase Ion Reactivity in Collision Cell ICP-MS/MS Analyses Through Theoretical and Experimental Analyses.

Khadouja Harouaka¹, Khadouja Harouaka¹, Kali Melby¹, Amanda French¹, Caleb Allen¹, Eric Bylaska¹, Richard Cox¹, Gregory Eiden¹, Maria Laura di Vacri¹, chelsie beck¹, brienne seiner¹, brian archambault¹, Eric Hoppe¹, Isaac Arnquist¹; ¹*Pacific Northwest National Laboratory*

The arrival of commercial atomic ICP-MS/MS eight years ago has made it possible for everyday analysts to utilize inline chemical resolution for the measurement of low levels of actinides and other analytes without the need for extensive sample preprocessing. The instrument utilizes inline gas phase chemistry to either react away the analyte from the mass region where isobaric interferences exist or remove the interference through a mass shift or collisional dissociation. The goal of our research is to understand and predict the reactivity of several commercially available gases with ions representative of the periodic table. Using such reactions during the measurement either eliminates or significantly reduces the sample purification required prior to making the measurement. Resolving a given interference depends on the selectivity of the reagent gas reactivity with the analyte of interest and interfering species. This approach can be used either as an alternative to, or as a complement to chemical separations ahead of the ICP-MS analysis, which makes it particularly useful for analyses like laser ablation ICP-MS/MS where limited sample preprocessing is possible. To understand gas phase ion-molecule reactivity in analytical instruments, we determine the instrument tuning dependence on the kinetic energy of the ions entering the collision cell using previously established models calibrated against guided ion beam experiments where the kinetic energy of the ion is known. We then compare the ion kinetic energies, the observed product ion formation and calculated Density Functional Theory (DFT) reaction enthalpies in order to gain a predictive understanding of what type of reactions are likely to proceed. Our current data sets show that the DFT derived reaction enthalpies correctly predict reactivity of ~90% of the ions studied using an Agilent 8900 ICP-MS/MS. Further questions remain regarding applying similar methodology to other ICP-MS/MS systems that may employ different collision cell multipoles and ion beam energies. We also briefly discuss incorporating the predictive construct we developed into an all-purpose data visualization, processing, and database type platform to aid unique method development for a variety of applications.

(ATOM-03.3) LIBS and Its Role in Nuclear Energy Applications

Supathorn Phongikaroon¹; ¹*Virginia Commonwealth University*

Laser-Induced Breakdown Spectroscopy (LIBS) is an elemental analysis technique, which is based on the emission from plasma generated by focusing a laser beam into the medium. This technology has been used in various mediums—solids, liquids (including molten metals), and gases—for different applications due to its robustness in elemental analysis with little to no required sample preparation. This attractive feature has brought attention to many researchers to study and explore in nuclear energy applications as LIBS

may provide a possible path in non-destructive assay detection and analysis and take the material accountancy to the next level. This talk aims to address challenges, successes, and approaches of LIBS in nuclear science, engineering, and technology – especially towards molten salt technology.

(ATOM-03.4) Laser Ablation Spectroscopy for Radioactive Plume Detection

Kyle C. Hartig¹, Kyle Latty, Emily Kwapis¹; ¹*University of Florida*

Laser-induced breakdown spectroscopy (LIBS) has emerged as an effective analytical technique for stand-off detection and real-time monitoring applications. In particular, the capability to promptly measure elemental and isotopic compositions of materials from standoff distances manifests unique benefits in nuclear and radiological settings where prolonged exposure carries risks. Previous works have demonstrated the applicability of LIBS for detecting actinide and fission products as solids and a limited number as gases; however, atmospheric plume tracking of fallout particulates reveals a challenge in low particle-loaded environments due to the infrequency of particle interactions with the focused pulsed-laser. This work aims to address atmospheric radiological material plume tracking using nanosecond LIBS (ns-LIBS), short-focus femtosecond (fs) LIBS (SF-LIBS), and fs-filament LIBS (FIBS) of fallout particle surrogates.

Samples are prepared through mixtures of 10,000 µg/ml ICP-grade solutions of Cs, Ce, and Fe at different ratios, where Ce is used as a surrogate for Pu with Fe as a common interference co-contaminant. A controlled multicomponent dry nanoparticle plume is generated in a sample chamber using a custom 3D printed drying apparatus that mixes a co-flow of compressed with an aqueous aerosol mist. It was determined that Cs nanoparticles can be easily detected at low concentrations among a diverse mixture of aerosol components due to the long-lived resonance emissions (beyond 300 µs gate delay for ns-LIBS) that outlasts both early background and interference signals from co-contaminants using both filaments and short-focused ns and fs pulses. Conversely, Ce experiences strong interference from Fe and N₂ transitions below 393 nm; thus, Ce I and Ce II spectra are investigated using multivariate analysis techniques across multiple emission lines.

This talk will focus on post-detonation nuclear debris detection and characterization through emission spectroscopy of excited atoms, ions, and molecules in laser-produced plasmas formed following ns, fs, and filament laser interaction. Perspectives on application to more complex species and measurement scenarios will be provided.

(ATOM-03.5) Spectroscopic Signatures and Oxidation Characteristics of Laser-produced Cerium Plasmas

Emily Kwapis¹, Kyle C. Hartig¹; ¹*University of Florida*

In the event of the detonation of a nuclear weapon, rapid and accurate knowledge characterizing the hostile environment is essential for the continuation of effective military operations with advanced situational awareness. This work aims to improve nuclear forensic capabilities by developing optical signatures of nuclear-relevant materials using standoff, optical detection techniques such as laser-induced breakdown spectroscopy (LIBS). LIBS

offers a robust, field-deployable analytical method capable of remote, isotopically resolved and phase identifiable multi-elemental detection with no sample preparation. The technique uses a high-powered pulsed laser to produce a luminous micro-plasma, or laser-produced plasma (LPP), which is then imaged to produce a spectrum of characteristic atomic and molecular emission lines characterizing a sample. LPPs have repeatedly been used as laboratory-scale surrogates for chemical detonations and nuclear explosions to study the highly complex properties of post-detonation fireballs. Nuclear fireballs and laser-produced plasmas are both known to be highly sensitive to their surrounding environment. Chemical reactions between the plasma and its environment result in the formation of molecular species, which can considerably impact fallout particle formation and debris distribution. The plasma response to reactive species such as oxygen can significantly influence the interpretation of optical signatures, where this behavior has repeatedly been demonstrated in the literature for uranium laser-produced plasmas. However, while extensive studies into the spectroscopic characterization and plasma chemistry of uranium have been performed, there has been little investigation into the properties of plutonium LPPs despite marked differences in chemistry between the elements. Therefore, this work seeks to address current gaps in the literature by conducting a comprehensive investigation into the physical phenomena and high-temperature gas-phase chemistry that drives the rapid evolution and complex interactions of plutonium nuclear fireballs with their surrounding environment. Laser-induced breakdown spectroscopy will be performed on a plutonium surrogate (cerium) to elucidate the effects of atmospheric conditions and oxidation reactions on nuclear optical signatures. Photographic techniques including time-resolved fast-gated imaging and shadowgraphy will also be conducted to explore the expansion dynamics and shock wave characteristics of laser-produced cerium plasmas. Multiphysics simulations will be provided alongside the experimental measurements to support explanations of the post-detonation phenomena.

22AWD06: AES Mid-Career Award Symposium Honoring Aditya Khair

Chair: Henry Chu

Co-Chair: Christopher Easley

(AWD-06.1) Electrohydrodynamic interactions of drops

Petia Vlahovska¹; ¹*Northwestern University*

The interaction of fluids and electric fields is at the heart of natural phenomena such as disintegration of raindrops in thunderstorms and many applications such as ink-jet printing, microfluidics, crude oil demulsification, and electrosprays. Many of these processes involve droplets and there has been a long-standing interest in understanding drop electrohydrodynamics. While an isolated drop in applied electric fields has been extensively studied, the behavior of many drops is largely unexplored. Even the pair-wise drop interactions have received scant attention and existing models are limited to axisymmetric and two-dimensional geometries. In three dimensions, the electrohydrodynamic interactions can be quite complex and non-trivial. For example, in an applied uniform electric field, instead of chaining along the field direction, drops can initially attract in the direction of the field and move towards each other, but then separate in the transverse direction [1]. Using a combination of numerical simulations based on a boundary integral formulation and an

analytical theory assuming small drop deformations, we study the dynamics of a drop pair in an applied uniform electric field at arbitrary orientation of their line-of-centers relative to the applied field direction. For identical drops covered with insoluble surfactant [2], we find that the surfactant weakens the electrohydrodynamic flow and thus dielectrophoretic interactions play more prominent role in the dynamics of surfactant-covered drops compared to clean drops. If drop conductivity is the same as the suspending fluid, a nondiffusing surfactant can arrest the drops' relative motion thereby effectively preventing coalescence. Drop dissimilarity can also have profound effect on the pair dynamics and results in droplets “swimming” either parallel or perpendicular to the applied field direction [3].

[1] Sorgentone et al. *J. Fluid Mech.* 914:A24 (2021)

[2] Sorgentone and Vlahovska, *Phys. Rev. Fluids*, 6: 053601 (2021)

[3] Sorgentone and Vlahovska, in prep.

(AWD-06.2) **Nonlinear Electrokinetic Flows in Insulator-based Dielectrophoretic Microdevices**

Xiangchun Xuan¹; ¹*Clemson University*

Insulator-based dielectrophoresis (iDEP) is an emerging technique for particle manipulation in microfluidic devices. It exploits insulating structures to generate electric field gradients for particle dielectrophoresis. However, the presence of these insulators, especially those with sharp edges, causes two nonlinear electrokinetic flows, which, if sufficiently strong, may disturb the otherwise linear electrokinetic motion of particles and affect the iDEP performance. One is induced charge electroosmotic (ICEO) flow because of the polarization of the insulators, and the other is electrothermal flow because of the amplified Joule heating in the fluid around the insulators. Both flows vary nonlinearly with the applied electric field (either DC or AC) and exhibit in the form of fluid vortices, which have been utilized to promote some applications while being suppressed in others. The effectiveness of iDEP benefits from a comprehensive understanding of the nonlinear electrokinetic flows, which is complicated by the involvement of the entire iDEP device into electric polarization and thermal diffusion. I will give in this talk a brief review of the works on the fundamentals and applications of ICEO and electrothermal flows in iDEP microdevices.

(AWD-06.3) **Harnessing Nonlinear Electrophoresis Effects**

Blanca H. Lapizco-Encinas¹; ¹*Rochester Institute of Technology*

Electrokinetics (EK) is a major branch of the field of microfluidics. Electrokinetic-based systems have been extensively employed for the analysis, separation, and purification of a wide array of bioparticles of interest, ranging from macromolecules to parasites. Electrokinetic effects are classified as linear and nonlinear, based on their dependence on the electric field magnitude. Linear EK effects, such as linear electrophoresis and electroosmosis, have been extensively studied and their mechanisms are well understood. Nonlinear electrokinetic effects, such as nonlinear electrophoresis, are still not fully

understood. The focus of this presentation, part of the Award Session in Honor of Professor Aditya Khair, is on the characterization and understanding of nonlinear electrophoresis effects in insulator-based electrokinetic (iEK) devices.

One of the most common formats of iEK devices is simple microchannels, made from glass or a polymer such as PDMS, that contain embedded insulating posts. These posts, which transverse the entire depth of the microchannel, function as 3D insulating structures that distort the distribution of the electric field within the channel. The presence of these 3D posts creates zones of higher field intensity when an electric potential is applied across the microchannel length. Nonlinear EK effects arise in these zones of higher electric field intensity and must be considered when studying the migration of target particles in iEK systems. The present work presents the combination of experimental and modeling work to characterize the effects of nonlinear electrophoresis, also called electrophoresis of the second kind, in polystyrene microparticles, bacterial and yeast cells in iEK devices. The nonlinear electrophoretic mobility is not independent of the electric field magnitude, which adds an extra layer of complexity to the understanding and application of this nonlinear phenomenon. Included in this presentation are our latest results on the characterization of the nonlinear electrophoretic mobility of polystyrene beads and cells. We will analyze the effect of particle/cell properties on their nonlinear electrophoretic mobility and demonstrate that good agreement is obtained between modeling and experimental results by including nonlinear electrophoresis effects.

Acknowledgments:

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(AWD-06.4) Delivering Colloids to Targets Hidden Within Porous Media

Todd Squires;

Transporting colloidal objects to specific locations within porous media is essential for many applications, including drug or cargo delivery, material fabrication, oil discovery and recovery, chemical and biological sensing, and remediation of polluted soils and groundwater aquifers. The delivery of small particles into porous environments remains highly challenging due to the low permeability to the fluids that carry these colloids. Even more challenging is that in most cases, the specific location of targets in the porous environment is not known and cannot be determined from the outside. Here, we demonstrate a two-step strategy to deliver suspended colloids to targets that are ‘hidden’ within closed porous media. The first step serves to automatically convert any hidden targets into solute-inertial ‘beacons,’ capable of sustaining long-lived solute outfluxes. The second step introduces the deliverable objects, which are designed to autonomously migrate against the solute fluxes emitted by the targets, thereby following chemical trails that lead to the target. Experimental and theoretical demonstrations of the two-step strategy lay out the

design elements required for the solute and the deliverable objects, suggesting a route to delivering colloidal objects to hidden targets in various environments and technologies.

(AWD-06.5) Diffusiophoresis-controlled Separation of a Colloid-electrolyte Suspension under Gravity and Solvent Evaporation

Henry C. W. Chu¹, Henry C. W. Chu¹, Jinjie Xu¹, Zhikui Wang¹; ¹*University of Florida*

Unidirectional drying of a colloidal suspension has been used widely for manufacturing materials, such as colloidal coatings. In this talk, we employ direct numerical simulations to analyze the advective-diffusive transport of a colloid-electrolyte suspension in a unidirectional drying cell under the influence of solvent evaporation, gravity, and diffusiophoresis. Solvent evaporation generates a flow to concentrate the colloids and electrolyte at the drying interface. The difference in the density between the colloids and the solvent induces a gravity-driven backflow that drives the suspension away from the drying interface. In addition, the concentration gradient of electrolyte near the drying interface could impact the colloid distribution drastically via diffusiophoresis. We report two new findings focusing on the impact of diffusiophoresis. First, when diffusiophoretic interactions between the colloids and solute are attractive, diffusiophoresis could significantly enhance the concentration of colloids near the drying interface and the magnitude of the gravity-driven backflow. Second, when diffusiophoretic interactions between the colloids and solute are repulsive, diffusiophoresis could weaken or even eliminate the focusing of colloids near the drying interface and the backflow, regardless of the density-difference between the colloids and the solvent or, equivalently, the strength of gravity. Our results enable systematically tailoring the separation of a colloid-electrolyte suspension by tuning the interactions between the solvent, electrolyte, and colloids under Earth's or microgravity, which is central to ground-based and in-space manufacturing.

22AWD07: SAS and Applied Spectroscopy William F. Meggers Award Symposium

Chair: Michael Blades

Co-Chair: James Piret

(AWD-07.1) Extracting Pertinent Information from Congested and Overlapped Vibrational Spectra using Filtering Techniques Like 2D-COS and Node Attenuation

Isao Noda¹; ¹*University of Delaware*

Vibrational spectra are sensitive not only to the molecular structure but also to the subtle influences of local submolecular environment. As such, they are very rich in information to the point that spectra are often highly congested with overlapping features to make the interpretation challenging. Tools are available to sort out and extract only the pertinent information by filtering out the unwanted portion of the spectra. Two such techniques, namely two-dimensional correlation spectroscopy (2D-COS) and node attenuation band narrowing method, will be discussed. Intensities of certain portions of spectra, which are under the influence of some perturbation applied to the system, vary in a concerted or synchronized manner if their spectral features originate from the same or strongly coupled moieties. The presence of such signal synchronization, or lack thereof, is the basis for 2D-COS analysis to classify spectral features by filtering out the interfering information. Node

attenuation is another filtering technique where broad hems of vibrational spectral band peaks, which often overlap with the neighboring peak profiles, are effectively attenuated. The result is an apparent band narrowing effect to resolve overlapped peaks. Unlike the other well established band narrowing techniques, such as Fourier self deconvolution and the second derivatives spectroscopy, node attenuation does not produce the unwanted and sometimes misleading negative side lobes. This lobe-free feature is especially important when used in conjunction with 2D-COS. Illustrative examples of the applications of these techniques are presented for the analysis of mixtures and multi-phase bioplastics made from vegetable oil.

(AWD-07.2) The Role of Raman Spectroscopy in Bioprocess Automation

Karen A. Esmonde-White¹, Maryann Cuellar¹, Justin Moretto¹, Ian Lewis¹;

¹*Endress+Hauser*

Raman spectroscopy is the leading PAT in upstream bioprocessing due to its robust, scalable, and proven technology. Raman provides deep levels of real-time process understanding and feedback control without destructive sampling. Raman is increasingly being utilized to overcome longstanding challenges faced when implementing QbD principles. Leveraging the increased process and product knowledge provided by Raman, significant progress has been made towards true real-time release. Integration of Raman feedback into the process control networks, has delivered strong returns for users in the form of increased process efficiency and product quality stability. However, legitimate concerns still exist within the industry regarding the complexity of powerful PAT integration, and its ease of use at various unit operations.

Most recently, Industry 4.0 principles and an increasing number of non-specialists using Raman are impacting its use and growth as a PAT. In addition to novel process implementations, we also highlight recent developments in Raman technologies in the areas of probe compatibility with micro/mini and single-use bioreactors, downstream process control, and integration to MVDA and automation platforms. These developments enable non-specialists to harness the power of Raman quickly and easily, as we move closer to the goal of turnkey PAT.

(AWD-07.3) Non-Destructive Infrared Spectroscopic Assessment of Developing Tissue

Nancy Pleshko¹, William Querido¹; ¹*Temple University*

In vitro development of cells and tissues (tissue engineering) for biological tissue repair has many challenges, one of which is assessment of the maturity of the developing construct/cellular mass. Recently, optical spectroscopic methods have been used for non-destructive evaluation of cells and tissues, including for engineered tissues. Infrared spectroscopy in the mid- and near infrared (NIR) frequency regions has been applied for direct assessment of matrix composition, and recently, for assessment of metabolic products in cell culture media that reflect construct development. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy was used to evaluate consumption of glucose and secretion of the metabolite lactate by cells in the culture media, processes that are associated with tissue development. Using a series of standards, it was shown that ATR-

FTIR data distinguished culture media with varying amounts of glucose and lactate. Specific absorbances of glucose at 1035 cm^{-1} and lactate at 1122 cm^{-1} strongly correlated with the concentration of these components. Tissue engineered cartilage constructs (TECs) were prepared using chondrogenic cells grown in hydrogels, and analyzed for cell viability, and formation of proteoglycan (PG, a major cartilage protein), and presence of metabolites in media. ATR-FTIR data obtained from cell culture media harvested during TEC development showed that the $1122/1035\text{ cm}^{-1}$ peak ratio was sensitive to distinguishing cultures with different construct numbers (1, 3 or 5 constructs/well) or to constructs at different developmental stages (3 or 5 weeks of culture). Furthermore, NIR spectroscopy also has also been used to directly assess construct development. Those studies found that data collected from live constructs with an NIR fiber optic probe showed that absorbances from collagen and/or proteoglycan (PG), at ~ 5940 and 5800 cm^{-1} , were sensitive to construct growth. Interestingly, the overall baseline offset of the raw NIR spectra strongly reflected matrix deposition, likely due to increased scattering in the constructs as they produce more matrix. Together, these data demonstrate that non-destructive applications of infrared spectroscopy can be extremely useful for *in situ* monitoring of the development of engineered constructs, which will aid in identifying individual samples that are optimal for tissue repair.

(AWD-07.4) **Countering COVID-19 through Better Diagnostics: On Label-free Spectroscopic Methods for Virus Detection**

Ishan Barman¹; ¹*Johns Hopkins University*

Widespread testing and isolation of infected patients is a cornerstone of viral outbreak management, as underscored during the ongoing COVID-19 pandemic. Here, we report a large-area and label-free testing platform that combines surface-enhanced Raman spectroscopy and machine learning for the rapid and accurate detection of SARS-CoV-2. Spectroscopic signatures acquired from virus samples on metal–insulator–metal nanostructures, fabricated using nanoimprint lithography and transfer printing, can provide test results within 25 min. Not only can our technique accurately distinguish between different respiratory and nonrespiratory viruses, but it can also detect virus signatures in physiologically relevant matrices such as human saliva without any additional sample preparation. Furthermore, our large area nanopatterning approach allows sensors to be fabricated on flexible surfaces allowing them to be mounted on any surface or used as wearables. We envision that our versatile and portable label-free spectroscopic platform will offer an important tool for virus detection and future outbreak preparedness.

(AWD-07.5) **Complexity in Raman Spectroscopy: The Curse of the n's with Samples from Biopharmaceutical Manufacturing**

Alan G. Ryder¹; ¹*National University of Ireland Galway*

Some of the difficulties associated with applying Raman spectroscopy in biopharma and developing robust Process Analytical Technologies (PAT) tools can be blamed on complexity. In the manufacturing of biologics nearly every sample is complex in terms of composition, structure, and/or physicochemical behaviour. This is true of raw materials,

cell culture media, bioprocess broths, and the final protein products. While using Raman spectroscopy for the analysis of materials encountered in small molecule manufacturing can be relatively straightforward, in Biopharma, three n's play a critical role in limiting what can be done. These are ns, the number of samples, na the number of atoms in a molecule, and nm the number of different molecules in the sample. These parameters coupled with the water factor, concentrations, scattering co-efficients, and some other physicochemical parameters impose some fundamental limitations on what is possible, on how you have to acquire Raman spectra in the first place, and also on how you can safely apply multivariate data analysis (chemometrics) to generate robust results and information. In this talk, I will show a variety of case studies where we have tried to apply Raman spectroscopy for the analysis of cell culture media, bioprocess broths, and other materials, illustrating the impact of the various factors.

22BIM03: Translation of Multimodal Imaging Technologies into Clinical Routine

Chair: Michael Schmitt

Co-Chair: Jürgen Popp

(BIM-03.1) Clinical Translation of Label-Free Multimodal Multiphoton Imaging for Point-of-Procedure Digital Pathology

Stephen A. Boppart¹; ¹*University of Illinois at Urbana-Champaign*

Innovations in biomedical imaging have historically led to discoveries in the life sciences and new detection and diagnostic technologies in medicine and surgery. Label-free intravital optical imaging and imaging of fresh, unstained, resected tissue specimens offers a wealth of new endogenous biomarkers and image-derived signatures for revealing the true colors of cancer and for diagnosing disease. Using new optical supercontinuum source technology and nonlinear optics to generate new excitation wavelengths, and by compressing and pulse-shaping the light stimulus in new ways, Simultaneous Label-free Auto-fluorescence Multi-harmonic (SLAM) microscopy can achieve fast simultaneous visualization of the rich intrinsic structural, molecular, and metabolic features within tissues. Two- and three-photon excitation of FAD and NAD(P)H, respectively, along with fluorescence lifetime measurements, enable imaging and quantification of metabolic activity, including determination of spatially-derived redox ratios within tissues, and spatial heterogeneity of activity and responses between cell populations. Second and third harmonic generation imaging enable microstructural visualization of collagen organization and cell/tissue interfaces, respectively. Quantitative machine/deep learning analyses of these high-content multi-dimensional datasets can subsequently be used to identify selective biomarkers for cancer. Specifically, tumor-associated extracellular vesicles (EVs) were detected *in situ* and label-free, and analyzed via their optical signatures and spatial distributions. Analysis of EV signatures from breast tissues from human subjects showed that EVs from the tumor microenvironment have unique optical signatures, in comparison to those from healthy subjects. This unique label-free imaging technology has been translated from the biophotonics imaging lab into clinical applications in surgery and pathology. The clinical demonstration of these optical biomedical imaging technologies with portable clinical imaging systems offers new paradigms for point-of-procedure diagnosis and guidance, as well as real-time assessment of culture-maintained excisional and core-needle

biopsy specimens, or “living biopsies”, following exposure to pharmacological treatment options.

(BIM-03.2) Raman Spectroscopy Devices for Intraoperative and in Situ Tumor Detection: Multicenter Retrospective Studies in Brain and Breast Cancer

Frédéric Leblond¹; ¹*Polytechnique Montréal*

Effective brain tumor surgery aims to remove tumor but not adjacent brain tissue. Cancer cells and associated structural and metabolic changes are often not visually distinguishable from normal brain during surgery. To address this, we conducted a multicenter study in 70 patients to test if a Raman spectroscopy device could detect the three most common intracerebral tumors during surgery: glioblastoma, meningioma and metastasis. The device was also used during breast conserving surgery to detect invasive ductal carcinoma in 21 patients. This is a new, robust, portable tool for in situ tumor detection that uses Raman spectroscopy and machine learning to detect biomolecular features associated with cancer status.

In vivo (in situ) Raman spectroscopy data were acquired from two neurosurgery centers (Montreal Neurological Institute Hospital, Mount Sinai Hospital in New York) using a handheld probe. In total, 2065 intraoperative spectroscopy measurements and colocalized tissue specimens were acquired from patients with glioblastomas, meningiomas, or brain metastasis. Measurements and corresponding biopsies were taken from the tumor bulk, tumor margins, and surrounding brain tissue. Biopsy sections were pathologically classified by tumor cell density. Spectral information and associated pathological labels were used to build machine learning classification models that were evaluated on independent subset of patients not involved in model training. The device achieved diagnostic accuracies of 87% for glioblastoma, 93% for brain metastases, and 96% for meningiomas. Similarly, more than 400 measurements were acquired in breast cancer patients demonstrating >90% accuracy to discriminate normal from cancer tissue. These data show that the device could be used intraoperatively to discriminate tumor from non-tumoral brain prior to resection.

Preliminary results will be presented in brain and breast cancer patients demonstrating the use of a new intraoperative line-scanning Raman spectroscopy instrument, effectively moving the technology beyond single-point detection to macroscopic imaging.

(BIM-03.3) Monitoring of Photodynamic Therapeutic Process of Cancer Cells with Pump-Probe Imaging Techniques

Zhiwei Huang¹; ¹*National University of Singapore*

(BIM-03.4) Detecting Real-Time In Vivo Esophageal Biochemical Changes in Pediatric Eosinophilic Esophagitis Using Raman Spectroscopy

Ezekiel Haugen¹, Andrea K. Locke¹, Girish Hiremath¹, Hernán Correa¹, Regina N. Tyree¹, Justin S. Baba¹, Anita Mahadevan-Jansen¹; ¹*Vanderbilt University*

Raman spectroscopy can be used for real-time *in vivo* identification of pediatric eosinophilic esophagitis activity.

Eosinophilic esophagitis (EoE) is an increasingly prevalent clinicopathologic condition. As an immune-mediated disease, it is characterized by an intense eosinophilic inflammation affecting the esophageal mucosa. An accurate diagnosis of EoE currently requires repeated upper endoscopy with multiple random esophageal biopsies due to the patchy nature of the disease. This approach is burdensome and risky, especially in children, and it prohibits timely diagnosis and monitoring of the condition. A safe point-of-care approach sensitive to EoE activity could improve patient care. Raman spectroscopy is an inelastic scattering technique sensitive to chemical bond information, providing a sample's biomolecular fingerprint. In this study, we investigated the utility of *in vivo* Raman spectroscopy (RS) for non-obtrusive point-of-care optical identification of EoE and employed nonlinear microscopy to further decipher the basis for the biochemical changes detected *in vivo*. A portable RS system coupled with a pediatric endoscope-compatible fiberoptic probe was used to acquire real-time spectra from the upper and lower esophageal mucosa of pediatric patients (between ages 6-17 years) undergoing upper endoscopy for clinical symptoms of EoE. Spectral analysis revealed unique alterations in bands associated with lipids (e.g., 1078, 1301, 1440, 2855 cm^{-1}) and water (3035-2680 cm^{-1}), which allowed for differentiation between active EoE and non-EoE controls. Additionally, differences in proteins (e.g., 1003, 1342, 2931 cm^{-1}) allowed for differentiation between active and inactive EoE. Nonlinear microscopy revealed changes to structural proteins (e.g., altered collagen fiber size) correlating with *in vivo* measurements. The results from this study demonstrate the potential of RS as a promising tool for real-time *in vivo* identification of EoE. The next step of this research will be the implementation of a trained model to investigate the accuracy of this novel approach for intra-procedure diagnosis and staging of EoE.

(BIM-03.5) Detection of Osteoporotic Related Bone Changes in Human Fingers Using ex vivo Raman Spectroscopy

Christine Massie¹, Andrew J. Berger¹; ¹*University of Rochester*

Detecting osteoporosis in human hands validates this region to non-invasively characterize bone health.

Osteoporosis is a bone disease that is diagnosed by measuring bone mineral density (BMD) through dual-energy X-ray absorptiometry. However, there is a need to obtain additional bone quality metrics to couple with BMD to accurately predict fragility fractures. Raman spectroscopy is a non-destructive optical technique that measures relative changes in bone

mineralization and matrix components. Several groups have demonstrated that Raman spectra from human femoral specimens' *ex vivo* provide information relevant to bone health. The purpose of this work was to validate that spectra from excised human finger bones give insight to bone health and could serve as a region of interest to perform transcutaneous measurements. Human hand cadavers were purchased from the Anatomy Gifts Registry (n = 12). The samples had various osteoporotic diagnoses, derived from conventional DXA scans from the wrists. Intermediate phalanges (IP), proximal phalanges (PP), and metacarpals (M) II-IV were excised and measured with Raman. The midshafts of the bones were measured using a 120 mW laser with a 5-minute exposure. For all bones (IP, PP, M) the phosphate mineral to matrix ratio was significantly decreased and carbonate substitution (carbonate/phosphate) was significantly increased in the osteoporotic samples ($p < 0.05$). Principal component analysis was performed and principal component 1 was significantly different between healthy and osteoporotic samples ($p < 0.05$). To further quantify differences in the matrix components, spectral regions commonly reported to be associated with matrix peaks (amide 1, amide 3, and CH_2) were modeled by sums of Gaussian-Lorentzian (GL) curves. For the CH_2 peak ($1360\text{-}1500\text{cm}^{-1}$) we observed the ratio of GL curves at 1438 cm^{-1} and 1450 cm^{-1} was significantly higher in the osteoporotic samples, which suggests a shift towards more adipose content in osteoporosis. Within the amide 3 region ($1220\text{-}1340\text{cm}^{-1}$) we observed the ratio of GL curves at 1300cm^{-1} and 1268cm^{-1} was significantly higher in osteoporotic samples. Within the amide 1 region ($1580\text{-}1700\text{ cm}^{-1}$) the ratio of 1658 cm^{-1} to 1636 cm^{-1} GL curves was significantly higher in osteoporotic bones. This evidence of osteoporotic changes in human hands validates the hand as a region of interest for transcutaneous Raman to characterize bone quality.

22CHEM05: Chemometric Opportunities in the Forensic Sciences

Chair: Igor Lednev

(CHEM-05.1) Fast Blue BB and 4-Aminophenol Colorimetric/Fluorometric Tests for the Differentiation of Hemp-Type and Marijuana-Type Cannabis and for the Determination of THC in Oral Fluid

Jose R. Almirall¹, Alexander G. Acosta¹, Ryan Capote¹, Nicole Valdes¹, Maira Kerpel dos Santos¹, Roberta Gorziza¹; ¹*Florida International University*

Two effective presumptive tests used to indicate hemp-type and marijuana-type cannabis the Fast Blue BB (FBBB) and 4-Aminophenol (4-AP) colorimetric tests were used to analyze 99 authentic marijuana samples and 93 authentic hemp samples. Red, Green, and Blue (RGB) scores were obtained for the chromophores (and fluorophore in the case of FBBB) from magnified images of the reaction products on the substrate. Linear Discriminant Analysis (LDA) and Data Driven-Soft Independent Modeling of Class Analogies (DD-SIMCA) models were constructed using the RGB scores. The LDA results showed that FBBB and 4-AP are effective at classifying THC-rich marijuana (THC:CBD >2) from hemp

individually but have a slightly higher specificity when both tests are used in combination. Marijuana samples with a THC:CBD below 2 were considered outliers for the SIMCA models. However, sensitivity and specificity above 95 % were achieved with the SIMCA models when these samples were excluded from the model. These observations and statistical results suggest that FBBB and 4-AP may be used either individually or in combination to reliably indicate hemp and marijuana when the THC:CBD is above two. We also report the miniaturization of the FBBB+ Δ^9 -THC reaction on a substrate along with the analytical figures of merit for a quantitative determination of Δ^9 -THC in plant extracts and in oral fluid. When the FBBB is reacted with Δ^9 -THC, a red chromophore forms that when irradiated with blue visible light (480- 540 nm) forms a complex that fluoresces. The fluorescence observed allows for more sensitive detection of the presence of the THC + FBBB complex. We report on the specificity and sensitivity (limit of detection), precision, and bias using an objective reading of fluorescence and color. The development of a quantitative method for the Δ^9 -THC reaction with FBBB is used as a proof of principle for the detection of other cannabinoids in complex matrices such as oral fluid OF, which can then be used for on-site detection of drugs to detect impaired driving, for example.

(CHEM-05.2) FTIR Spectroscopy in Forensic Applications Advanced by Machine Learning Approaches: Making Data-Driven Decisions

Lenka Halámková¹; ¹*Texas Tech University*

Fourier transform infrared (FT-IR) spectroscopy is used throughout forensic laboratories for many applications. FT-IR spectroscopy can be useful with ATR accessories in forensic analysis for several reasons. It provides excellent data quality combined with high reproducibility, with minimal user-induced variations and no sample preparation. ATR FT-IR spectra provides structural information about a sample based on the selective absorption of radiation in the mid-infrared region. The examination of fingernail and toenail specimens has been typically used for drug and alcohol testing. Compared to soft tissues, nails are highly stable, easy to decontaminate, simply to collect, and easy to ship and store. The process of sampling is non-invasive, non-destructive and they retain a very discrete, detailed record of information about originator, such as genetic inheritance, diet, disease, drug, medication use, etc. Spectra from heterogeneous biological systems, including the integumentary system, can be associated with hundreds or thousands of biomolecules. The nail matrix of keratin possesses a complicated structure with captured circulating metabolites whose presence may vary in space and time depending on context and history. We developed new approach by making use machine-learning tools (ML) to leverage the potential and enhance the selectivity of the instrument, create classification models, and provide invaluable information saved in human nails with statistical confidence. The primary goal of this research was to determine whether an individual's age, sex, race, smoking and alcohol status can be determined based on ATR FT-IR spectra collected from their nail clippings using ML computational framework. Such a relationship would allow forensic investigators to glean additional information about a person of interest using physical evidence found at a crime scene.

(CHEM-05.3) **Chemometrics for Extraction Useful Information from Raman Data: A Data Analysis Protocol**

Thomas W. Bocklitz¹, Thomas W. Bocklitz¹; ¹*Leibniz Institute of Photonics Technology*

Raman spectroscopic techniques are increasingly utilized in various disciplines such as biology and medicine, as well as in forensics. This increased number of applications is connected with the improvement of the Raman setups, like for example portable or handheld devices, and computational data science methods, like chemometrics and machine learning. The latter mentioned data science methods can extract (high-level) information in the application context from subtle differences in the Raman spectra. The high-level information depends on the task and the sample, e.g., the prediction of sample properties and the detection of the presence and absence of substances.

The Raman effect is a weak process, and hence the Raman spectra might be overshadowed by artifact contributions, which makes it necessary to investigate the whole data life cycle of Raman data from its generation, via the data modeling to the archiving. The most critical points within the life cycle for Raman data are the experiment design, the sample size planning, the data pre-treatment, the data pre-processing, chemometric and machine learning based data modeling, model transfer methods and transfer learning. All procedures are sequentially combined in a data pipeline, which standardizes the data and extracts reliable high-level information from the Raman data. The sequence of data science methods needs standardization itself, and we suggested a first Raman data analysis protocol. This protocol represents a first step to construct a standardized data analysis pipeline for real-world applications of Raman spectroscopy [1]. The presentation will focus on the comparability of Raman spectra between instruments and labs, which was studied in a European ring trial [2].

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References:

[1] S. Guo, *Nature protocols* 2021, 16, 5426

[2] S. Guo, *Analytical Chemistry* 2020 92, 15745

(CHEM-05.4) **Rapid Detection and Classifications of Pathogens using Raman Spectroscopy and Artificial Intelligence.**

Siva Umamathy¹, Sanchita Sil¹, Dipak Kumbhar¹, Dhanya Reghu¹, Divya Shrunagar¹; ¹*Indian Institute of Science*

The detection of pathogens using rapid, non-invasive, accurate and sensitive technology is the need of the hour in forensics and defence. The conventional method of identification of micro-organisms primarily includes culture-based, immunoassay, biosensor, and polymerase chain reaction. The main disadvantage of these conventional techniques is they are time-consuming, labour intensive and expensive. Other detection techniques include biosensors which measures optical, electrochemical, thermometric, and piezoelectric signal variation with the attachment of biological components, such as enzymes, nucleic acid,

antibodies, etc. Though biosensors are sensitive and rapid, they are very unique to each species, making it more complex in field applications.

Vibrational spectroscopic techniques have advantages over these conventional microbiological approaches toward the identification & detection of pathogens. Because the molecular structure of the biomolecules in these pathogens is unique, Raman spectroscopy investigation can yield distinct spectral fingerprints. Therefore, these spectral markers can be used for tracking diseases, studying the effectiveness of drugs in cells and tissues, identification of pathogens and many other biological processes.

Conventional Machine learning algorithms used in chemometrics work well with a few subclasses of bacteria or pathogens only. If the number of classes is increased, they tend to lose accuracy and robustness. Therefore, one needs to use multiple algorithms to check which one is performing well. Therefore, there is a need to develop new AI models to handle rising datasets with a large number of classes with better accuracy and robustness. In recent times, deep learning, a subset of machine learning, is prevailing over conventional machine learning algorithms in the Raman spectroscopy domain with an increased number of classes. Convolutional Neural Network (CNN) is a deep learning-based classification approach that collects and learns important features from unknown input to determine which class it belongs to. AI algorithms were developed to accurately classify pathogen categories with more than 98% accuracy despite having spectral similarity. We have shown that Raman spectroscopy could classify highly similar bacterial strains with the help of AI techniques.

(CHEM-05.5) DART-High Resolution Mass Spectrometry (DART-HRMS) for Identification of the Resource That Necrophagous Insects Fed on

Samira Beyramysoltan¹, Amy M Osborne¹, Jennifer Y. Rosati², Rabi A. A. Musah¹;

¹University at Albany-SUNY, ²John Jay College of Criminal Justice

Mass spectrometry in combination with chemometrics result in identification of resource that insects fed on

Necrophagous insects that have colonized decomposing remains can play a critical role in forensic investigations, as their species identity can be used to estimate post-mortem interval. However, insect evidence has the potential to reveal much more about the circumstances associated with a death. In this regard, it would be useful to be able to determine whether retrieved insect evidence fed on human verses animal remains from direct examination of the insects, as this may be of relevance to a crime. Recently, the mass spectral chemical fingerprints of insect species at various life stages, acquired by DART mass spectrometry, were shown to enable accurate determination of species identity. We report here an investigation of whether these DART-MS chemical profiles can also reveal the food resource ingested by the flies. Eggs of three species (*C. vicina*, *L. sericata*, and *P. regina*) were reared on five resources: beef liver, pork chop, dog feces, chicken breast, and decaying tilapia. The

emergent subsequent life stages were collected and stored in 70% aqueous ethanol until analysis. The DART-HRMS data of these samples were acquired from analysis of their aqueous ethanol suspensions. The data were binned and scaled, and the resulting matrix was explored by the multifactor method ANOVA simultaneous component analysis to reveal variations in the chemical profiles that were a function of species and resource type. A fusion of partial least square-discriminant analysis and principal component analysis-discriminant analysis was performed to create a discriminative model for the reliable identification of not only species, but also food resource using selected m/z values. The performance analysis of the method showed 95% and 52% accuracy by five-fold cross validation for larvae and adult respectively. The results illustrated that the chemical profiles of adult samples were more influenced by external conditions, in comparison with larva samples, which affected identification accuracy. Therefore, analysis of the larval life stage, which is the most commonly encountered insect form in forensic investigations, can be used not only for species determination, but also for determination of resource substrate.

22FORENS02: Food Forensics

Chair: Luis E. Rodriguez-Saona

(FORENS-02.1) Development of a Handheld Sensor Technology for Real-time Measurement of Food Quality Traits

Christopher Ball¹, Luis E. E. Rodriguez-Saona¹; ¹*The Ohio State University*

Researchers at The Ohio State University have developed a handheld sensor based on near-infrared spectroscopy that can measure key food quality traits in real time with accuracy comparable to benchtop instruments. This technology uses a miniaturized spectrometer in combination with a sample rotation stage and multivariate calibration algorithms to analyze processed or intact food and agricultural products. A custom-built application running on a commercial tablet both controls the operation of the sensor and analyzes the measured spectra. This talk summarizes the development of this sensor device and recent tests involving a variety of agricultural products.

(FORENS-02.2) NMR Techniques in Edible Oil Analysis and Authentication

Emmanuel Hatzakis¹, Emmanuel Hatzakis¹; ¹*Ohio State University*

NMR spectroscopy is a powerful tool for the compositional analysis of complex mixtures, such as foods and plants. Its combination with chemometrics allows for spectral pattern comparison and for the identification of biomarkers. Here, we present the application of NMR methodologies for the analysis and authentication of edible oils. More specifically, we focus on the analysis of avocado oil and its differentiation from other edible oils including olive, canola, high-oleic (HO) safflower, HO sunflower and soybean oil, using high-field and low-field NMR. Further, we used NMR to differentiate between virgin and refined coconut oils. In all cases NMR was able to distinguish between different products. This is of specific importance, especially for low-field NMR which is a more affordable and user-friendly technique that fits well in an industrial environment. Finally, we used NMR to

distinguish between olive oils produced from four super-high-density cultivars, namely, Arbequia, Arbosona, Koroneiki and Sikitita and we compared its performance with standard targeted analysis of fatty acids and triglycerides.

(FORENS-02.3) Detection of Some Common Food Adulterations in Türkiye Using Vibrational Spectroscopy

Huseyin Ayvaz¹; ¹*Canakkale Onsekiz Mart University*

Economically-motivated adulteration of foods, the shortage of high-value food sources, and globalization of the food markets have made confirming food authenticity a growing challenge for the food industry worldwide. Based on a survey, the number of food fraud cases in Europe per year has more than doubled since 2016. Regarding the recent suspicions of food fraud, the European Union Commission sent about 200 requests to authorities in EU countries such as Spain and Italy and 100 requests to non-EU countries, including China and Türkiye. In Europe, fats in dairy products and oils (particularly extra virgin olive oil) are the most debated and top notified products for food frauds, along with seafood.

Accordingly, this presentation will emphasize some of the relevant studies conducted in the rapid detection of food adulterations in Türkiye using near-infrared (NIR) and mid-infrared (MIR) spectroscopies, along with some of the work performed utilizing Raman and laser-induced breakdown (LIB) spectroscopies. As complementary or alternative to traditional benchmark food analytical methods, these spectroscopic methods have gained paramount importance as prompt and dependable approaches to food authenticity assessment and have been positioned as attractive technologies offering high-speed, versatility, unique capabilities, convenience, and low-cost monitoring of food characteristics. In line with the most frequently adulterated food products in Europe, this presentation will emphasize some of our studies conducted in the rapid detection of food adulterations in a variety of foods, including milk, yogurt, cheese as well as olive oil, hazelnuts, mussels, and flours in Türkiye using NIR, MIR Raman and LIB spectroscopies.

(FORENS-02.4) Developing Surface Enhanced Raman Scattering-based biosensors for In Situ Detection of Agriculturally Relevant Targets

Lyndsay Kissell¹, Pietro Strobba¹; ¹*University of Cincinnati*

The first demonstration of iMS sensor-gel networks for *in situ* detection of agriculturally relevant targets.

The current standard for detecting agriculturally relevant analytes requires analysis by a centralized lab. Field-deployable methods could disrupt the current paradigm in agricultural analysis (e.g., plant diseases and contaminants detection), leading agricultural processes towards sustainable and efficient practices. To this end, new technologies capable of

sensitive, accurate and field-deployable analysis are needed for detection of these analytes *in situ*. In this work, we will discuss the development of a sensing platform that can be deployed for *in situ* detection of infected or contaminated plants. The platform uses an homogeneous surface-enhanced Raman scattering (SERS) sensing mechanism based on the inverse molecular sentinel (iMS) approach to detect agriculturally relevant targets. These sensors can be incorporated in an agar gel network and on optical fibers for use *in situ*. These sensors can be tailored to detect plant disease biomarkers (DNA), plant pathogens (viral RNA), and/or small molecule contaminants (pesticides). Finally, the SERS sensing mechanism could lead to highly multiplexed detection of a range of targets in a single, rapid measurement.

(FORENS-02.5) **Real-Time Screening of Major Cannabinoids Content in Hemp by a Novel Handheld FT-NIR Spectroscopic Approach**

Siyu Yao¹, Christopher Ball¹, Gonzalo Miyagusuku-Cruzado¹, M. Monica Giusti¹, Luis E. E. Rodriguez-Saona¹; ¹*The Ohio State University*

A novel designed handheld FT-NIR sensor allowed the rapid screening of major cannabinoids in hemp.

Plants belonging to *Cannabis sativa* are classified into two groups, marijuana and hemp. Hemp is valued for its medicinal compounds in contrast with marijuana that is used recreationally for its psychoactive properties. Legalization of hemp in the 2018 Farm Bill removed the crop from the Controlled Substances Act. To be considered as hemp, cannabis plants should have Δ^9 -tetrahydrocannabinol (Δ^9 -THC) level < 0.3%. Accurate and reliable determination of Δ^9 -THC and cannabidiol (CBD) is of great economic importance to stakeholders in the cannabis supply chain. Our objective was to employ a novel NIR spectroscopic for real-time detection and quantification of major cannabinoids in hemp through spectral signature profiles. A total of 91 hemp samples from certified online vendors (n=54), OARDC Lab (n=34), and a local hemp grower (n=6) were collected and homogenized (~4g) for this study. Reference data of major cannabinoids contents was determined by uHPLC-MS/MS with an ESI interface. Spectral data were collected by micro-electro-mechanical system-based handheld FT-NIR spectrometer and combined with the reference data to generate partial least square regression (PLSR) models. uHPLC-MS/MS analysis showed that two samples had over 0.36% of Δ^9 -THC, and 68% (34 out of 50) of online-bought hemp samples were incompliant with their total CBD content declaration. PLSR prediction models showed strong correlation (Rpre=0.91-0.95) and low standard error of prediction (SEP = 0.02–0.61%). Our prediction models were superior or comparable to results from benchtop infrared systems. Our technology can quantify CBD and THC in short measurements (~15 sec) displaying the results wirelessly in an app operated by a tablet. Farmers, hemp buyers, CBD product manufacturers, and researchers would benefit greatly

from a low-cost, sensor technology that provides field-deployable, real-time, in situ CBD assays.

22IR10: Coblenz, New England SAS, and New York/New Jersey SAS Celebrating Success of Nurturing Talent in Vibrational Spectroscopy

Chair: John Wasylyk

Co-Chair: Larry McDermott

(IR-10.1) The Impact of Hot-Carriers on Surface Enhanced Raman Spectroscopy

Chelsea Zoltowski (Goetzman)¹, Zac D. Schultz¹; ¹*The Ohio State University*

Changes in the SERS signal can elaborate on transient species formation associated with plasmonic activity.

Plasmonic nanostructures have paved the way for the development of surface enhanced Raman spectroscopy (SERS); a technique that takes advantage of the Raman signal specific to the molecular vibrational modes. SERS enhances the Raman signal up to 10^9 -fold allowing for lower limits of detection. Through the illumination of the nanostructure with a laser, a localized surface plasmon resonance (LSPR) is excited and further enhances the electric field at the surface of the nanostructure. While the excitation of the LSPR enhances the Raman signal, it can also generate hot carriers that cause the formation of transient species that can change the Raman signal. Photoproducts have been reported for various nanostructures in different SERS experiments and can include cross-linking/dimerization, fragmentation, and radical formation. Understanding the parameters and occurrences of transient species will allow for the ability to prevent them when not desired and generate them for further applications. Previously, our group has reported on radical formation with the amino acid tryptophan. This work will use changes in the SERS signal to elaborate on the conditions and dynamics of these radical formation reactions associated with the plasmonic activity of nanostructures.

(IR-10.2) Sensitive Nitric Oxide detection using Interferometric Cavity-Assisted Photothermal Spectroscopy

Daide Pinto¹, J.P. Waclawek¹, Stefan Lindner¹, Harald Moser¹, Giovanna Ricchiuti¹, Bernhard Lendl¹; ¹*TU Wien*

Photothermal Spectroscopy (PTS) is an indirect technique that measures thermal effects on a gas sample induced by modulated photon absorption and molecular non-radiative relaxation. The periodically absorbed energy is released in the form of heat, producing a local gas expansion. The magnitude of such effects scales linearly with the optical power of

the excitation source, taking full advantage of QCL sources. In Interferometric Cavity-Assisted Photothermal Spectroscopy (ICAPS) a Fabry-Pérot Interferometer (FPI) and a probe laser are used as optical transducer for refractive index variations, induced by gas thermal expansion. The FPI reflectivity changes as the refractive index of the filling medium is perturbed by the gas expansion. The thermal effect scales with the concentration of the analyte and is ultimately detected as a change in reflected intensity.

A DFB-QCL emitting at 1900 cm^{-1} was used to target the R(6.5) absorption line of nitric oxide (NO) and induce the thermal expansion. The QCL wavelength was scanned across the absorption line, and a $2f$ -wavelength modulation spectroscopy ($2f$ -WMS) approach was used by dithering the current at f_{mod} . A fibre-coupled diode laser (1552 nm) was coupled to the FPI and the reflected intensity was collected on a photodetector (APD). The highest sensitivity is achieved when the probe wavelength (λ_p) is tuned to an inflection point (IP) of the interferometric fringe. For this purpose, we employed WM of the probe via a modulation (at frequency f_p) of its current. The APD signal was demodulated by lock-in amplifier (LIA-1) at $2f_p$ which was fed to a PID, acting on the DC offset of the probe to keep the $2f_p$ signal at the zero-crossing (which corresponds to the IP). This approach ensures stable and efficient detection of photothermal effects, being essential for ICAPS operation. The PTS signal was obtained by LIA-2, demodulating the APD signal at $2f_{\text{mod}}$. A noise equivalent concentration of 1.4 ppm was achieved with 3 sec time constant, corresponding to a normalized noise equivalent absorption of $5 \cdot 10^{-6}\text{ cm}^{-1}\text{ W Hz}^{-1/2}$.

(IR-10.4) Mid-Infrared Biomarkers of Lupus Nephritis Using Optical-Photothermal imaging

Chalapathi Gajjela¹, Rohith Reddy¹, Chandra Mohan, Anto Crosslee, Camille Artur;

¹*University of Houston*

Mid-infrared spectroscopic imaging has been used in various applications, from archeology to material characterization to cancer grading. However, it suffers from low spatial resolution due to long mid-infrared wavelengths. Optical photothermal infrared imaging (O-PTIR) overcomes some of these limitations and provides an order of magnitude improvement in spatial resolution. Histopathologic assessment has been the current standard of care for diagnosing lupus nephritis (LN) but often has a poor inter-pathologist concordance of less than 50%. Despite being crucial, the markers derived from traditional immunohistochemical staining do not provide a complete picture of the disease heterogeneity and phenotype. MIRSI provides both biochemical and morphological information without stains and is a promising technique for a potential increase in the diagnostic efficacy of LN. MIRSI can quantitatively measure the changes in morphology and distribution of the kidney's intrinsic biomolecular constituents, such as collagen, lipids, and nucleic acids, contributing independent information to histopathologic assessment. In our current work, we use murine model to assess the diagnostic contribution of O-PTIR imaging by comparing spectral differences between Wild-Type (WT) mice and mice with LN. Several characteristic mid-IR spectral markers were identified; particularly, statistically significant differences among the average spectra at peaks relating to collagen, nucleic acid, and lipids collected from these two datasets.

(IR-10.5) **How to Survive as an Early Career Researcher**

Mike George¹; ¹*University of Nottingham*

How to survive at the start of your career can be challenging. Indeed, how to survive is not easy at all stages of career progression. Nobody plans their career. Your career is made up of the choices which you make from the opportunities that present themselves and the only advice is never to regret decisions that you make as you cannot change them. This lecture is aimed at encouraging early career researchers. It will highlight how I have used a 'recipe for scientific survival', which I believe was first presented by John Rabolt at the 1985 Coblentz Award, and use research examples drawn from research to highlight how this 'recipe for scientific survival' can be used.

22LIBS02: Advanced Approaches I

Chair: Vassilia Zorba

(LIBS-02.1) **Nanoparticle Enhanced Laser Induced Breakdown Spectroscopy for Biological Applications**

Alessandro De Giacomo¹, Alessandro De Giacomo¹, Marcella Dell'Aglio², Rosalba Gaudiuso¹; ¹*University of Bari*, ²*CNR-NANOTEC*

Although NELIBS has been demonstrated in several applications [1], one of the most interesting uses of such technique is the elemental analysis of biological samples. As a matter of fact traditional LIBS is not that sensitive with biological sample because of the high ionization energy of the matrix elements (C, N, H) that tends to quench the plasma and in turn it requires several accumulations in order to reaching a high S/N. The latter requires amounts of samples that are not always available in real applications or that are not convenient with respect to traditional techniques. NELIBS allows to bypass mostly these inconveniences with a single shot analysis, because of the more effective conversion of the laser energy into sample atomization and further excitation. In this lecture some examples of elemental analysis on biological samples, including liquids, proteins [2] and tissues [3] will be discussed as well as the use of NELIBS for retrieving information on the structure of NP-protein complex structures [4].

[1] M. Dell'Aglio, R.A. Rifai, A. De Giacomo, *Spectrochim. Acta B* 148 (2018) 105-112.

[2] A. De Giacomo, C. Koral, G. Valenza, R. Gaudiuso, M. Dellaglio, *Anal. Chem.* (2016), 88 (10), pp. 5251-5257

[3] M. Dell'Aglio, Z. Salajková, A. Mallardi, R. Mezzenga, L. van't Hag, N. Cioffi, G. Palazzo, A. De Giacomo, *Spectrochim. Acta B* 155 (2019) 115-122.

[4] M. Dell'Aglio, Z. Salajková, A. Mallardi, M.C. Sportelli, J. Kaiser, N. Cioffi, A. De Giacomo, *Talanta* (2021), 235, art. no. 122741

(LIBS-02.2) **Back Deposition of Titanium Oxides under Laser Ablation of Titanium: Simulation and Experiment**

Igor B. Gornushkin¹, Vadim Veiko², Julia Karlagina², Andrey Samokhvalov², Dmitry Polyakov²; ¹*BAM Federal Institute for Materials Research and Testing*, ²*State University for Information Technology, Mechanic and Optic*

Understanding of laser processing of titanium dental implants is important for human health

Titanium is widely used in medicine for implants and prostheses, thanks to its high biocompatibility, good mechanical properties, and high corrosion resistance. Pure titanium, however, has low wear resistance and may release metallic titanium into surrounding tissues. Structuring and coating its surface with oxide layers are necessary for high wear resistance and improved biocompatibility. In this work, a combination of theoretical and experimental methods was used to study processes responsible for deposition of titanium oxides during ablation of titanium in air.

The deposition process was modeled via the Navier-Stokes equations that accounted for the material removal and accumulation of the deposit on the ablation surface. The chemical part was based on the equilibrium model embedded into the hydrodynamic code. Simulations showed that the most active zone of production of condensed titanium oxides were at plasma periphery whereas a zone of strong condensation of titanium metal was above the molten pool.

In experiment, a pulsed Yb fiber laser was scanned across a titanium surface. The temperature and composition of the plasma were inferred from plasma emission spectra. The post-ablation surface was analyzed by SEM, TEM, STEM, AFM, and XRD.

The developed model well reproduced the main features of experimental data. It was concluded that the deposition of condensed metal oxides from the plasma is a principal mechanism of formation of nanoporous oxide layer on the metal surface. The method of surface structuring and modification by nanosecond laser ablation can be developed into a useful technology that may find applications in medicine, photonics, and other areas.

(LIBS-02.3) Multi Sensor Laser Ablation Analysis of Complex Samples

Jhanis J. Gonzalez¹, Charles Sisson, Chunyi Liu², Vassilia Zorba¹, Dayana Oropeza, Jose Chirinos¹, Richard Russo¹; ¹*Lawrence Berkeley National Laboratory*, ²*Applied Spectra, Inc. / Lawrence Berkeley National Laboratory*

Tandem laser ablation-based systems that combine both LIBS and LA-ICP capabilities were developed to exploit some unique advantages for the analysis of solid samples. Highlighted in this presentation is the flexibility that multiple independent but simultaneous sensors help to ensure that the sensitivity needs for elements of interest are met. This type of instrumentation addresses the needs of the scientific community for new technology to analyze heterogeneous materials faster and simpler with improved sensitivity and precision

(LIBS-02.4) Using LIBS to Characterize High Entropy Alloys for Extreme Environments

Prasoon K. Diwakar¹, Bharat Jasthi², Nicholas E. Pugh¹; ¹*South Dakota School of Mines*, ²*South Dakota School of Mines and Technology*

Novel application of LIBS for high entropy alloys

Multi-principal element alloys (MPEAs) and high entropy alloys (HEAs) are a class of material that are now increasingly being used in extreme environments due to their superior properties. When operating in conditions that can cause the material to fail in unusual ways, it is important to know what is happening with the structure of the material in real-time. In this study MPEA AlNbTaTiZr was synthesized using arc melting and laser induced breakdown spectroscopy (LIBS) was used to analyze and determine various properties of the MPEA. When analyzing LIBS spectra of MPEA, presence of various lighter elements can lead to matrix effect. It is important to determine when and where a matrix effect can occur with various laser-based techniques, such as LIBS. The samples that were created and tested were either made from high-purity metals (Al, Nb, Ta, Ti, and Zr), or high purity equimolar concentrations of AlTi, AlNbTi, AlNbTiZr, and AlNbTaTiZr. It will be shown that there was a matrix effect present when titanium was added to aluminum and vice-versa but to a lesser extent when other components were added. Using the LIBS spectral analysis, various materials properties were extracted including microhardness. Further details and mechanisms of LIBS analysis MPEAs high entropy alloys will be presented.

(LIBS-02.5) Laser-induced Breakdown Spectroscopy for Analysis of Molten Salts

Daniel Diaz¹, David Hahn¹; ¹*University of Arizona*

Novel in-situ detection of off-gassed species from molten salts at temperatures as high as 505°C.

Laser-induced breakdown spectroscopy was used to analyze off-gassed species from samples of pure sodium nitrate (NaNO₃) and pure and doped lithium chloride-potassium chloride eutectic (LKE) at temperatures between 320 and 505 °C. LKE samples were doped with CeCl₃ and NdCl₃ at concentrations as high as 10% w/w Ce and Nd. Up to 15 g of sample were deposited in a custom-made crucible and heated up to 505 °C. The system mainly consisted of a crucible with controlled and preheated Ar atmosphere for sample melting, and a LIBS analysis chamber with optical access. A tubular 3-kW electric furnace, three 0.4-kW electric coil heaters, and one 0.83-kW electric heating tape were used to heat up, respectively, the crucible and sample, tubing and fitting accessories, and the LIBS analysis chamber. The off-gassed species were analyzed in the LIBS chamber while kept at 450 °C. The LIBS system consisted of a 1064-nm, 6-ns, 180-mJ, Nd:YAG laser; a Czerny-Turner spectrometer (300-mm focal length); and an ICCD camera (1064 × 256 pixels). Emission lines from Ca (Ca II 393.66 and Ca II 395.85 nm), Ce (spectral range 375-495 nm), Na (Na I 588.99 and Na I 589.59 nm), and Nd (spectral range 375-495 nm) were investigated. Plots of analyte emission intensity versus time were used for analyte detection.

The detection of Ca (present in the raw LKE compounds) at a concentration of about 150 ppm demonstrated the satisfactory detection capability of this LIBS prototype, particularly for analytes with high-intensity emission lines. Detection of analytes with atomic lines of lower emission intensities was more challenging due to the high-intensity interfering Ar lines. The detection of analytes after relatively consistent times and temperatures agreed the expected sample phase change (i.e., from solid to liquid), especially for NaNO₃, for which Na emission lines were detected at temperatures close to the melting point. Future work on chemical analysis of off-gassing from molten salts will include the study of other salts and analytes at the same and higher temperature ranges. This work was supported by the U.S. Department of Energy under Award Number DE-SC0021919.

22PAT02: SAS PAT Technical Section: PAT in BioPharma and Pharma

Chair: Dan Hill

Co-Chair: Hossein Hamedi

(PAT-02.1) Enhanced Process Understanding of Lentiviral Manufacturing by Real-Time Raman Spectroscopy

Erin Masucci¹, Erin Masucci¹, Karin M. Balss¹, Brynne Jensen, Carl Rafferty, Ryan Morrison, Emily Curtis¹; ¹*Janssen*

Raman spectroscopy is a proven process analytical technology (PAT) used to monitor cell culture processes in biopharmaceutical manufacturing. Raman spectra from inline Raman probes provide real-time chemical fingerprints of the manufacturing process. Here we provide an example of Raman monitoring during upstream cell culture for the bioreactor stage of lentivirus production at different manufacturing scales. In order to understand the biochemical changes during lentivirus production, Multivariate Partial Least Squares (PLS) regression models were developed to create a relationship between Raman spectra with offline reference values for metabolites, biomass, and yield. The models were useful to in actively monitoring batch progression. In combination with multivariate analysis, predictions of yield are demonstrated. The ability to monitor and predict outcomes in real time helps guide both process optimization and scale-up and technical transfers.

(PAT-02.2) Raman Backed Model Predictive Control: Strengthening Raman's Utilization in Small-Scale Bioprocess Development

Matthew Demers¹; ¹*Amgen Inc*

Process analytical technologies (PATs) have the potential to play a critical role not only in the manufacturing but also the development of biologic processes. The two PATs studied for this work are Raman technology and Model Predictive Control (MPC). Separately, Raman technology and MPC have shown benefits within upstream PD such as effective process monitoring, and process control of cell culture. The work covered in this presentation aims to address two major pursuits within the biotechnology industry, improving speed to market and increasing process automation and digitalization, by

combining Raman and MPC technologies. The combination of these two technologies lead to a better glucose feeding strategy which allows for a more advanced processes to be designed in a quicker timeframe with greater understanding. In previous work, MPC was effectively used by itself to optimize glucose concentrations resulting in increased final day titer production, but it required manual administration of glucose feeds which proved to be a time sink for staff working in the small-scale labs. With the closed loop control, staff were freed up to perform more value-added work while maintaining the effectiveness of MPC. The Raman had also been used in previous experiments to control glucose however the control strategy lacked the complexity to optimize glucose concentrations like MPC is capable of. The closed loop control MPC based feeding strategy used linear and non-linear models to determine an optimal glucose target concentration for feeding and then used Raman predicted glucose values at the time of feeding to calculate the appropriate feed volume. Implementing this control strategy affected cell culture productivity and final product quality attributes. Further work needs to be done to improve the consistency of the automated feed. With more development, these technologies can be used to quickly design optimal upstream cell culture processes based on cell specific inputs.

(PAT-02.3) Multi-Attribute Raman Spectroscopy (MARS) for Monitoring Product Quality Attributes in Formulated Monoclonal Antibody Therapeutics

Bingchuan Wei¹, Bingchuan Wei¹; ¹*Genetech*

Rapid release of biopharmaceutical products enables a more efficient drug manufacturing process. Multi-attribute methods that target several product quality attributes (PQAs) at one time are an essential pillar of the rapid-release strategy. The novel, high-throughput, and nondestructive multi-attribute Raman spectroscopy (MARS) method combines Raman spectroscopy, design of experiments, and multivariate data analysis (MVDA). MARS allows the measurement of multiple PQAs for formulated protein therapeutics without sample preparation from a single spectroscopic scan. Variable importance in projection analysis is used to associate the chemical and spectral basis of targeted PQAs, which assists in model interpretation and selection. This study shows the feasibility of MARS for the measurement of both protein purity-related and formulation-related PQAs; measurements of protein concentration, osmolality, and some formulation additives were achieved by a generic multiproduct model for various protein products containing the same formulation components. MARS demonstrates the potential to be a powerful methodology to improve the efficiency of biopharmaceutical development and manufacturing, as it features fast turnaround time, good robustness, less human intervention, and potential for automation.

(PAT-02.4) Rapid Amino Acid Quantitation by an Integrated CE-MS Analyzer

Kenion H. Blakeman¹, Hannah Wilker¹, Colin Gavin¹, Ji Young Anderson¹, Scott Miller¹; ¹*908 Devices*

Automated quantitation of amino acids by an integrated CE-MS analyzer is discussed.

Characterizing cell culture nutrients such as amino acids in cell culture media is critical for maximizing cell growth and improving key quality attributes of the cells. Traditional methods for amino acid analysis in process analytical chemistry such as HPLC suffer from complexities spanning sample preparation through data analysis. A custom microscale ion trap mass spectrometer (MS) operating at high pressures coupled to a microchip capillary zone electrophoresis (CE) analyzer platform has been developed to simplify this process. This analyzer performs amino acid quantitation including routine performance qualification checks in under 10 minutes per sample without active user monitoring or data processing.

The CE-MS system including the CE separation and microscale ion trap technology will be discussed in the context of developing a system capable of automated calibration and data processing. Specific components that will be highlighted include vacuum pumps, ion tap design, and automated fluidic handling with an autosampler. Simplification of sample preparation to a simple spin, dilute, and analyze the sample will be presented. Finally, automated data generation and automated green light/red light data analysis will be discussed.

To validate the analytical performance of the amino acid analyzer, a combination of amino acid standards and commercially available cell growth media were analyzed. Sigma AAS18 standard was diluted 25x to 500x and analyzed across 4 CE-MS analyzers. Quantitative accuracy and precision across this 20x calibration range will be discussed. Medium 199, IMDM, and RPMI-1640 medium were also analyzed across the 4 CE-MS analyzers at 10x to 25x dilution factors. Finally, to highlight the robustness of the automated quantitation to common sample interferences encountered in cell culture media such as salts, antifoams, and proteins, these species were spiked into commercial CHO medium at biologically relevant concentrations. Median % RSD values in the 10% range will be shown for the amino acid standards and commercially available media.

(PAT-02.5) In Situ Raman Spectroscopy for Real Time Detection of Cysteine
Justin Lomont¹, Joseph P. Smith²; ¹*Merck*, ²*Merck & Co*

We herein report in situ Raman spectroscopy for real time monitoring of cysteine

Cysteine serves a wide range of important biological and chemical functions and may have an association to neurodegenerative disease and cancer. Rapid, accurate analytical methods for cysteine detection are thus highly desirable. In this work, we report an investigation into the utility of in situ Raman spectroscopy as a Process Analytical Technology (PAT) for real

time monitoring of cysteine. Cysteine concentrations are tracked in real time using Raman spectroscopy across a range of pharmaceutically-relevant concentrations, demonstrating the capability of Raman spectroscopy detection for in situ cysteine monitoring. The concentration range over which this analytical methodology can be applied is successfully established. As such, the results herein serve as a proof-of-principle investigation to demonstrate and evaluate the capabilities of a real time Raman spectroscopic approach for in situ cysteine detection, thus informing the range of important chemical and biological processes to which this approach can be applied. To the best of our knowledge, this is the first report of in situ Raman spectroscopy for real time monitoring of dynamically changing cysteine process concentrations.

22PMA09: Small Molecule Profiling

Chair: Katherine Hollywood

Co-Chair: Royston Goodacre

(PMA-09.1) Good, Fast and Cheap in Metabolomics and Synthetic Biology, Choose One?

Karl E V Burgess¹, Joan Cortada Garcia¹, Georgie Barrett¹, Tessa Moses¹, Jennifer Haggarty²; ¹*University of Edinburgh*, ²*University of Glasgow*

The fourth industrial revolution is the development of biology as an industrial tool. Engineering biology has made great strides in the automated synthesis of gene constructs and strains, indeed the development of automated facilities, such as the Edinburgh Genome Foundry, allowing thousands of strains to be synthesized per week creates an unprecedented capacity for metabolic engineering. However, testing and analysis lag far behind in throughput and breadth of metabolite coverage, with most facilities relying on laborious and time consuming liquid chromatography and liquid chromatography-mass spectrometry experiments. Once high-performing strains are selected, scale-up from shake flask to fermenter is fraught with difficulty, with most fermentation development being performed on an ad-hoc basis.

My lab focuses on the development of new metabolomics workflows supporting synthetic biology. We present two platforms for improved strain and bioprocess development: RHIMMS - an ultra-high performance hydrophilic interaction liquid chromatography-high resolution ion mobility mass spectrometry method allowing a global metabolomics analysis to be performed in 3.5 minutes injection to injection; and RTmet - instrumentation and methodology for high temporal resolution on-line mass spectrometry monitoring of fermentations.

In this presentation, we discuss the parameters of the RHIMMS method and its applications in industrial biotechnology, and the value of RTmet's highly resolved time courses in fermentation analysis.

(PMA-09.2) NMR-based Isotope Editing, Chemoselection and Isotopomer Distribution Analysis in Stable Isotope Resolved Metabolomics

Andrew N. N. Lane¹, Penghui Lin, Teresa Fan¹; ¹*University of Kentucky*

NMR is a very powerful tool for identifying and quantifying compounds within complex mixtures without the need for individual standards or chromatographic separation. Stable Isotope Resolved Metabolomics (or SIRM) is an approach for following the fate of individual atom from precursors through metabolic transformation. However, extracts of cells or tissue give rise to very complex NMR spectra, which is exacerbated by the presence of NMR-active stable isotopes. While multidimensional NMR experiments may partially overcome the spectral overlap problem, additional tools may be needed to determine site-specific isotopomer distributions. NMR is especially powerful by virtue of its isotope editing capabilities using NMR active nuclei such as ¹³C, ¹⁵N, ¹⁹F and ³¹P to select those molecules in a complex mixture that contain these atoms, and provide direct information about which atoms are present in each identified compound and their relative abundances. The isotope-editing capability of NMR can be also be employed to select for those compounds that have been derivatized at particular functional groups with an NMR-active stable isotope enriched reagent, leading to considerable spectral simplification. I will review and present new approaches to isotopomer analysis by NMR, and methods of chemoselection of different functional groups for spectral simplification, using recent examples from cancer metabolism.

(PMA-09.3) Stable Isotope Tracing of Nutrients From Consumption to Energy Production in Humans: A Step Towards Understanding Metabolism and Developing Therapeutic Interventions in the Fanconi Anemia Population

Lindsey Romick-Rosendale¹, Lindsey Romick-Rosendale¹, Sara Vicente-Munoz, Thomas Galletta, Suzanne Summer, Stella Davies; ¹*Cincinnati Children's Hospital Medical Center*

Fanconi anemia (FA) is a rare inherited syndrome characterized not only by a high incidence of bone marrow failure, but also a high risk of hematopoietic and epithelial malignancies. Additionally, studies have found that 22-38% of persons with FA are underweight, suggestive of increased risk of developing malnutrition. Many persons with FA experience gastrointestinal problems, early satiety, poor appetite and poor weight gain. It is likely that persons with FA are more prone to being malnourished because of the metabolic burden related to their disease. Studies have also found that more than 50% of persons with FA have depleted muscle mass. Malnutrition, in an immunocompromised population, can contribute to increased morbidity and mortality. As both standards of care and accurate body composition assessments have improved, more studies are needed to address the association between metabolism and poor growth and development in the FA population.

We first used steady-state NMR-based metabolomics to identify metabolic abnormalities relating to FA. We noted a number of metabolic abnormalities based on both urine and plasma profiles of persons with FA compared to non-FA matched controls, including accumulation of ketone bodies in the urine, dysregulation of compounds relating to thyroid hormone synthesis and acetaldehyde metabolism, and increased presence of free amino

acids suggesting an increased push towards protein degradation in this population. With the advent of stable isotope-resolved metabolomics (SIRM) in recent years, researchers are now able to track the metabolic fate of individual small molecules in living organisms. We employed NMR-based SIRM techniques using orally administered uniformly ^{13}C -labeled glucose under fasted conditions to determine impairments in glucose uptake and processing. We paired this approach with bionutritional assessments, indirect calorimetry and screening assays to assess hormone production and secretion to obtain a more robust picture of the overall impact of metabolic dysfunction in the FA population. With the goal to improve the clinical care affecting the growth and nutritional status in the FA community, our work aims are focused on developing treatment targets and nutritional supplement protocols that will improve the overall physical and emotional health and body image of persons with FA.

(PMA-09.4) Direct Nanoelectrospray Ultra-high Resolution Mass Spectrometry in Stable-Isotope Labeled Metabolomics

Richard M. Higashi¹, Richard M. Higashi¹, Teresa Fan¹, Andrew N. N. Lane¹; ¹*University of Kentucky*

Metabolism is a complex network of coupled and sometimes circular chemical reactions that transfers **sub**structures of molecules, thus metabolite **sub**structures must be tracked to decipher networks in e.g. cancer metabolic reprogramming. Stable isotope addition to biological systems provides the experimental means, and stable isotope resolved metabolomics (SIRM) distinguishes pathways and functions, among even otherwise identical metabolites, by tracking the provenance of **sub**structures, using NMR and ultrahigh resolution (UHR) MS. Thus, SIRM achieves functional proteomics by conducting simultaneous, untargeted, coupled enzyme assays *in situ*, revealing up/down regulation of pathways in metabolic reprogramming. To accommodate SIRM, we routinely use direct nanoelectrospray (nESI) ultra-high resolution (UHR) MS, using, as an example, lipids extracted from A549 cells in 3D culture treated with $^{13}\text{C}_6$ glucose and analyzed using an Advion Nanomate interfaced to Thermo Fusion Lumos with 500,000 resolution setting and UVPD. Without any chromatography, nESI-UHR-MS³ via UVPD photodissociation could establish near-complete resolution of lipid isobaric isomers, including the acyl chain positions of *sn1* and *sn2* plus the unsaturation positions within the acyl chains. More importantly, for SIRM the number and substructure location of ^{13}C atoms to *sn* locations and within acyl chains can be determined for glycerophospholipids. A “deep dive” example will be presented using $^{13}\text{C}_9$ isomer of PC, where there appears to be no ^{13}C incorporation beyond C₁₆ for the acyl chains, that is, from the elongation pathways, so one interpretation is that ^{13}C was incorporated only via FASN. In turn, because $^{13}\text{C}_6$ glucose was the principal external carbon source in this experiment, elongation for the acyl chains in this lipid used pre-existing carbon source(s). Such data yields pathway activity hypotheses, including insight to elongase and desaturase activity.

(PMA-09.5) High Throughput Analysis and Ultra-Small Volume Detection of Biological Samples Using Droplet Imbibition Mass Spectrometry

Taghi Sahraeian¹, Abraham Badu-Tawiah¹; ¹*The Ohio State University*

High throughput analysis and ultra-sensitive nanoliter volume detection of biological samples by Mass Spectrometry

We have developed a new droplet imbibition mass spectrometry (MS) platform that enables high-throughput analysis and ultra-small volume detection of complex mixtures. This becomes important where many different samples are required to be analyzed in short amount of time. Our method achieves this by eliminating sample preparation whilst also enabling an array of samples to be detected. Our droplet imbibition source uses an array of optimized-tip glass capillaries to contain the samples. The tip of each glass capillary is sampled by charged microdroplets derived from an electrospray ionization (ESI) emitter, which picks up (imbibe) the analyte from the glass tip and transfers the desorbed analyte directly to the mass spectrometer inlet. Associated advantages with this platform include: 1) high-throughput analysis: by separating analyte solution from the ESI spray solvent and sampling with charged microdroplets, complex mixtures can be effectively analyzed without sample pretreatment; 2) ultra-small sample analysis: by sampling the tiny glass capillary tip with high speed (100 m/s) microdroplets ($< 5 \mu\text{m}$), only small volume of the analyte is desorbed. The sampling rate for droplet imbibition was experimentally determined to be in 1.5 nL/min at 150 psi N_2 nebulizing gas pressure, as opposed to 1-100 $\mu\text{L}/\text{min}$ for ESI and $\sim 100 \text{ nL}/\text{min}$ for nano-ESI. This represents more than two orders of magnitude reduction in sample consumption, compared with nESI, which currently represents the most efficient means of analyzing small sample volumes; 3) surface effects: by using high speed microdroplets to sample analytes in close proximity of the MS inlet, the desorbed analyte has limited time to diffuse into the core of the droplet. This ensures that majority of the desorbed analytes reside at the surface of fast-moving droplet, eliminating ion suppression effects caused by competition between analytes to occupy droplet surface. This enhanced surface effect dramatically increases the sensitivity of the droplet imbibition MS. This was shown by constructing calibration curves for cocaine in human urine and whole blood with 2 and 7 pg/ml limits of detection (LOD), respectively. Sensitivity of droplet imbibition was compared with ESI which showed 4 orders of magnitude increase in sensitivity for droplet imbibition experiment.

22SPECIAL03: Celebrating Peter Griffiths' 80th Birthday

Chair: Ian Lewis

(SPEC-03.1) Microfluidic Modulation Spectroscopy: A New Approach for Probing Protein Secondary Structure

Don Kuehl¹, Don Kuehl¹, Eugene Ma²; ¹*Cerno Bioscience*, ²*RedShiftBio*

Protein characterization is critically important in the development, formulation, and manufacturing of biologic pharmaceuticals. Characterizing protein secondary structure is used to help understand drug stability, aggregation, similarity, and efficacy. Traditional methods include ultraviolet circular dichroism (UV-CD) and FTIR. Unfortunately, UV-CD is unsuitable for measuring the typical high protein concentrations present in final formulations and is also relatively insensitive to the all-important beta-sheet structures, an important indicator of aggregation. FTIR is restricted to mainly high protein concentrations (typically 10-150 mg/mL), requires extremely short pathlength transmission cells (typically 4-8 μ m) which can be tedious to work with, and is difficult to automate.

Microfluidic Modulation Spectroscopy (MMS) is a new measurement technique designed to address many of these limitations. First, a tunable, continuous mode, quantum cascade mid-IR light source is used to scan across the amide I band from about 1720 to 1580 cm^{-1} . This extremely bright source is up to 1000 times brighter than a typical FTIR light source which allows for the use of relatively long (25 μ m) transmission cells which are relatively easy to work with and significantly increases the measurement sensitivity. To minimize the effects of drift (source, temperature, detector, electronics, etc.), a specially designed transmission flow cell rapidly alternates the sample and a buffer reference into the beam path using microfluidic methods at rates as high as 10Hz. The differential nature of the measurement also increases the measurement repeatability and minimizes the interference problems of water vapor which contains strong absorbance bands across the amide I band.

MMS improves the sensitivity of IR protein measurement to cover a concentration range of 0.1-200 mg/mL, suitable for the diverse concentration range encountered from development through manufacturing. The flow cell design lends itself to automating the measurement technique which increases the sample throughput substantially as well as and improving the ease-of-use.

In this presentation, we will describe in detail the implementation of MMS and discuss some of the challenges encountered while developing the technique. Examples of real-world applications in the pharmaceutical industry will illustrate the use of MMS to investigate aggregation, biosimilarity, stability, and quantitative analysis of a variety of protein-based therapeutics.

(SPEC-03.2) Laboratory Mentoring to become a President

Christine Pharr¹; ¹*Mount Mary University*

As a nontraditional chemistry graduate student and mother of two small children, becoming a university president is not a typical career consideration. Yet, lessons learned in designing experiments, problem-solving, writing professional journal articles and presenting at national conferences lend themselves well to this future career choice, provided and they are packaged with high expectations and supportive mentorship. This presentation will tell the story of how Peter Griffiths mentorship, lead this graduate student to a very successful career in academic leadership.

(SPEC-03.3) Data Preprocessing Method for the Analysis of Spectral Components in the Spectra of Mixtures.

Richard Jackson¹, Richard Jackson¹, Qian Wang², John Lien³; ¹*Galaxy Scientific, Inc.*,
²*Galaxy Scientific Inc.*, ³*Operant, LLC*

This presentation will describe a data preprocessing algorithm that can be used to mitigate the effects of interfering spectral components when the goal is to detect the spectrum of unknown components in a mixture of known components or to verify the presence of suspected components in the spectrum of a mixture of known components. The algorithm is both relatively simple and applicable to a wide range of problems in spectroscopy. The range of applicability can be increased by combining the method with other data preprocessing methods, for example derivative spectra, and can also accommodate variability in the spectra of one or more of the known components. Examples of the application of the algorithm to real problems will be given for near-infrared analysis of antibiotic drug formulations inside gelatin capsules, Raman analysis of injectable drugs, and mid-infrared analysis of atmospheric pollutants.

(SPEC-03.4) A Brief History of Optical Metrology

Chris Manning¹, Andrew Helbers¹, Mathew Philippou¹, Yoav Kargon¹, Alexander Bianco¹, Tyler Morgus¹; ¹*Thorlabs, Inc.*

In the 220 years since Young's experimental demonstration of the wave nature of light and the almost simultaneous discovery by Herschel of infrared light, wavelength references have become increasingly important. Optical metrology is the application of light to measurement of distance and time. At least as early as 1867 [added in proof: 1859?], Maxwell was aware of the possibility of using yellow light from sodium vapor as a standard of distance. Subsequently, both the Système International (SI) meter and the SI second have been defined in terms of electromagnetic transitions. A few decades after Maxwell's recognition, Michelson applied interferometric measurements of the light from gas discharge lamps to the problem of defining the meter. More recently, Michelson's interferometer design has been used extensively for Fourier transform-infrared (FT-IR) measurements, which [NAIP: generally] falls into the class of calibration-transfer interferometry. The accuracy of a laser wavelength can be transferred from a reference channel of an interferometric spectrometer to a signal channel containing an unknown spectrum. The Connes used gas discharge lamps in their pioneering spectral measurements of another planet. Had helium-neon lasers been available at the time, they would have been greatly preferred. Just over a decade later, HeNe lasers figured prominently in the advent of commercial FT-IR spectrometry. While helium-neon lasers still are used in some FT-IR spectrometers, semiconductor lasers have significantly altered the market. Diode lasers are much more compact, produce less waste heat and have dramatically longer lifetimes. Unfortunately, it has been difficult to provide diode lasers with the intrinsic wavelength reference provided by the helium-neon gain curve. Matching the long coherence length of the helium-neon laser also is challenging. A particularly appealing type of semiconductor device for FT-IR metrology is the vertical cavity surface emitting laser (VCSEL) geometry. A number of companies now are manufacturing FT-IR instruments

based on VCSEL wavelength references. This presentation will illuminate some of the golden threads of frequency calibration from inception to the present day.

(SPEC-03.5) Mid-Infrared Sensors - From Emerging Tool to Enabling Technology
Boris Mizaikoff¹; ¹*Ulm University and Hahn-Schickard*

Vibrational spectroscopies - and especially infrared spectroscopy - play an increasingly important role in modern biagnostics. This has led to the evolution of mid-infrared spectroscopy & photonics from an emerging tool in the clinical/medical domain to an enabling technology.

With applications ranging from non-invasive exhaled breath analysis to in-vivo assessment of cartilage damage, mid-infrared (MIR; 3-20 μm) photonics ranges among the most flexible molecular sensing platforms nowadays available. In particular, with the emergence of quantum and interband cascade laser technology, the on-chip hybridization and/or integration of entire MIR sensing devices is on the horizon ultimately leading to IR-lab-on-chip systems. The inherent molecular selectivity of MIR signatures enables studying small molecules (e.g., volatile organic compounds; VOCs) in the gas phase, as well as biomacromolecules (e.g., proteins) in the liquid phase at unprecedented detail in a label-free and non-destructive fashion.

Last but not least, the combination with advanced multivariate data evaluation and deep learning algorithms facilitates analyses in real-world complex mixtures of biomedical and clinical relevance. The discussion of latest MIR photonic technologies in this presentation we will be augmented by highlight applications underlining the utility of next-generation MIR photonics.

And by the way - Happy Birthday, Peter!

22AES05: AES Lifetime Achievement Award Session Honoring Adrienne Minerick

Chair: Christopher Easley

(22AES05-01) Ion Gradients in Dielectrophoretic Microdevices: Spatiotemporal Development and Impacts on Cells

Adrienne R. Minerick¹, Azade Tahmasebi, Sanaz Habibi, Jeana Collins, Ran An; ¹*Michigan Technological University*

Bioparticle motion and cell behaviors within dielectrophoretic microdevices have been studied in a range of electric fields and charging frequency ratios, but the parameter space at or below the charging frequency has not been fully explored. The charging frequency is the transition from well-established electric double layers (EDL) to partially formed EDLs. Cell membrane stability and cell viability correlate to EDL stability; assumptions that these properties are constant and at steady-state are foundational to many applications of dielectrophoresis. Our group previously explored micro-scale spatiotemporally controllable ion and pH gradients using reaction-free electrokinetic techniques in aqueous and methanol microfluidic environments; the gradients were attributed to Faradaic reactions

and electromigration mechanisms. Separately, artifacts of these gradients were quantified via human red blood cell (RBC) lysis and crenation under a limited range of frequency and amplitude conditions. More recent work in our lab has further characterized pH and ion gradients at or below the charging frequency in an aqueous solution to better complete the frequency and electric field density parameter space. The goal is to fully characterize microdevice-imposed artifacts on cells so clinical diagnostic devices can operate under highly reproducible conditions. This talk will explore two microdevices with T-shaped and star-shaped microelectrode geometries. Results reveal dependencies on hafnium oxide (HfO₂) coatings of microelectrodes that provide insights into the role of Faradaic reactions, while frequency and applied voltage dependencies provide insights into the influence of ion electromigration rates under different field conditions. Lastly, low concentrations of surfactants illustrate protective properties for cells adapting to these microdevice-imposed ion gradients. This work more deeply explores the frequency/electric field/solution parameter space so that microdevice artifacts can be controlled or eliminated from chemical and biological detection applications in diagnostic devices.

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(22AES05-02) **Multiplexed Traumatic Brain Injury (TBI) Assays using Particle Capture and Sorting**

Mark A. Burns¹, Mark A. Burns¹, Frederick Korley, Alyse Krausz, Sanaz Habibi;

¹*University of Michigan*

Traumatic brain injury (TBI) is a major cause of mortality and disability among children and young adults globally. In the United States alone, an estimated 2.5 million people sustain a TBI annually, and 50,000 of these individuals die of their injuries. In emergency-care practice, life-saving clinical decisions that influence the diagnosis and treatment of severe TBI are typically made within a period of minutes, and there is a critical need to develop point-of-care (POC) devices to aid in this important decision-making process. Measuring protein biomarkers such as glial fibrillary acidic protein (GFAP) in blood, plasma, or serum samples would be particularly useful as such tests have recently received FDA approval. In addition, studies show that combinations of more than one biomarker for TBI diagnosis have better diagnostic accuracy in comparison to a single biomarker. A quick, simple, and accurate blood test for multiple markers would enable caregivers to decide such things as the need for CT scans in patients with suspected mild TBI, potentially reducing healthcare costs and unnecessary neurological imaging.

We have developed a microfluidic device that can quantify the presence of multiple protein biomarkers in blood for detecting and monitoring TBI injuries. The device uses a variable-height channel to capture and segregate beads according to their size for simple multiplexed assays. Each size bead binds a unique protein using a sandwich antibody pair: a surface-bound antibody that binds to a TBI associated protein and a detection antibody with a linked quantum dot that binds to a different region of the protein. The beads are loaded into a ~1 cm long variable height channel that is ~10-20 microns high at the inlet and ~1-5 microns high at the outlet. Each set of beads is trapped at the position where the channel height equals its diameter. The resulting trapped beads show up as fluorescent bands indicating which proteins were present in the sample. The test uses only ~100 μL of whole blood or serum and can produce a response in as little as 15 minutes, providing clinicians actionable information regarding the condition of their TBI patients that was previously inaccessible.

(22AES05-03) Experiments in Mass Transfer: Inductive Heating and Micro Ring-Disk Electrodes

David O. Wipf¹, David O. Wipf¹, Timothy J. Wipf¹; ¹*Mississippi State University*

Teasing out electrode reaction mechanisms requires every more ingenuity in producing illuminating data. In addition to the normal process of applying potential or current programs to the electrode, the use of scanning electrochemical microscope (SECM) methods can be employed to enhance mass transfer through positive feedback and additionally, examine reactions at localized regions of surfaces, for example, at high-index microcrystallites or grain boundaries. Additionally, one might develop scanning probes that are multivalent, measuring multiple analytes or phenomena simultaneously. Finally, one might add additional parameters to the measurement, such as temperature or magnetic field.

Here we describe a novel high frequency induction heating instrument that can wirelessly heat ultramicroelectrodes without interrupting electrochemical measurements made using an ordinary potentiostat. The new heating method can be applied to working electrodes made of various materials (i.e. Pt, Au, Ni, carbon-fiber, and carbon-steel). A compact heating-generator housing contains all components necessary for sine wave generation as well as amplification and transformation of the heating power. The new arrangement yields high temperature cyclic voltammetric signals for the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox systems and others. Rapid heating (< 1 s) of several types of ultramicroelectrode materials (i.e., Pt, Au, Ni, carbon fiber, and carbon-steel) is demonstrated. Using this method, temperature-pulse voltammetry (TPV) acquired during the cooling transient following a heating pulse permits acquisition in a single scan of voltammetric data over a wide range of temperatures (ambient to >80 °C), with obvious advantages for understanding electrode kinetics and mechanisms.

In addition, we describe methods to prepare well-characterized and geometrically controlled microscopic ring-disk electrodes. In analogy to the well-known, large, rotating ring-disk electrodes, these electrodes can be used to examine multiple chemical species during SECM imaging and, in particular, examine products of chemical reactions accompanying electron

transfer. The electrodes prepared have disk radii of $< 5 \mu\text{m}$, with ring-disk spacings of a micrometer, making them ideal for SECM use.

(22AES05-04) **Presentation Title TBD**

Tayloria Adams¹; ¹*University of California, Irvine*

22ATOM05: Food

Chair: Todor I. Todorov

(ATOM-05.1) **Occurrence and Quantification of Toxic Elements in Ready to Eat Baby Foods**

Patrick J. Gray¹; ¹*US Food and Drug Administration*

Toxic elements such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) can enter the food supply because of past agricultural practices, industrial waste, leaching of toxic elements from containers or utensils that contact foods, and plant uptake from geological sources. The US Food and Drug Administration (FDA) routinely monitors toxic elements in the food supply. Special emphasis is placed on foods commonly consumed by young children because their smaller body sizes and metabolism make them more vulnerable to the harmful effects of these contaminants.

The FDA action plan, *Closer to Zero*, identifies actions the agency will take to reduce exposure to toxic elements from foods eaten by babies and young children. As part of the cycle of continual improvement, the FDA gathers data on the occurrence and amounts of toxic elements found in baby foods on the US market. These data are used to inform agency decisions and action levels.

A large, non-targeted survey was conducted in 2021 to estimate the current range of toxic element concentrations in ready to eat baby foods. Four hundred samples were purchased both online and in brick-and-mortar retail. Samples were analyzed by acid assisted microwave digestion and ICP-MS with a strong emphasis on lowering detection limits. This presentation includes results of this survey, and will include important lessons learned that were found to improve method blank cleanliness and detection limits. Observed relationships (or lack thereof) between toxic element concentrations and brands, ingredients, food packaging materials, and organic growing conditions will be presented.

(ATOM-05.2) **Analysis of Toxic and Other Trace Elements in Baby Foods by ICP-MS**

Chady Stephan¹, Liyan Xing, Aaron Hineman¹; ¹*PerkinElmer Inc.*

Commercial baby foods are the main source of nutrients and energy for many children around the globe, and therefore, the quality and safety of baby foods is extremely important during these crucial development stages. Arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) are toxic elements. The Food and Drug Administration (FDA) and the World Health Organization have declared them to be harmful to human health, particularly to babies and children who are undergoing neurological development.

In March of 2021, the US House of Representatives introduced the bill “ Baby Food Safety Act of 2021” (1) to regulate the presence of toxic elements in infant and toddler food products. Following this act, the U.S. FDA published a ‘Closer to Zero’ plan, which outlines steps that the agency will take over the next three years (and beyond) to reduce the toxic elements in foods eaten by babies and young children to as low levels as possible.

ICP-MS is a powerful elemental analysis technique with multi-element detection capabilities, low detection limits, high speed of analysis and wide linear dynamic range, making it the clear choice for total elemental analysis for baby food products.

As with all analytical techniques, it is affected by plasma and matrix-based polyatomic and doubly charged ion interferences. PerkinElmer’s NexION ICP-MS is equipped with Universal Cell Technology (UCT) that can operate in Collision mode with Kinetic Energy Discrimination (KED) and Reaction mode with Dynamic Bandpass Tuning (DBT) and Standard mode to mitigate/remove the interferences effectively with versatility.

This work describes a procedure for the analysis of toxic elements: Cd, Hg, Pb, and As, and other trace elements: Cr, Mn, Fe, Ni, Cu, Zn, Mo, and Tl, in baby foods following US FDA EAM 4.7 using the Titan™ MPS microwave digestion system for sample digestion and the NexION ICP-MS 2000 for sample analysis.

(ATOM-05.3) Lowering Detection Limits for Arsenic Speciation in Baby Food

Kevin Kubachka¹, Sean D. Conklin¹, Dominique Stutts¹, Kimberly Beers²; ¹*US Food and Drug Administration*, ²*Public Health Laboratory*

Recently, media outlets have reported that certain ingredients in baby foods were found to contain high concentrations of elements such as arsenic, cadmium, lead, and mercury. These findings were followed by a congressional committee report, subsequent proposed legislation (*The Baby Food Safety Act of 2021*), and an FDA action plan titled *Closer to Zero (C2Z)*. The anticipated levels of detection are near or below that of currently utilized FDA methods and therefore would require method modifications.

One analyte in particular, inorganic arsenic (sum of arsenite and arsenate), is determined using speciation analysis by liquid chromatography with inductively coupled plasma mass spectrometry. While current FDA methodology for arsenic speciation in juice reaches proposed detection limits, further improvements to a similar method for rice and rice product analysis were needed. This presentation discusses the changes to the FDA’s arsenic speciation methods (known as EAM 4.10 and 4.11) to reach low detection limits.

(ATOM-05.4) Detection of Endogenic Copper Nanoparticles in Streptomyces Coelicolor and its Effect on Secondary Metabolism

Paula García Cancela¹, Nathaly González Quiñónez¹, Mario Corte Rodríguez¹, Ángel Manteca fernández¹, Jörg bettmer¹, Maria Montes-Bayon¹; ¹*University of Oviedo*

Evaluation of nanoparticle formation after copper exposure and its link to antibiotic production in *Streptomyces*.

The modulation of the secondary metabolism in *Streptomyces* induced by copper is well known. However, the possible formation of endogenous copper nanoparticles and its relation to the production of antibiotics in one of best studied representatives of the genus, *S. coelicolor*, has never been established. In this work, the incorporation of Cu in individual spores of *Streptomyces coelicolor* at different Cu exposure concentrations was performed and evaluated using single cell-inductively coupled plasma-mass spectrometry (SC-ICP-MS). The quantitative results revealed incorporation of this metal that increases, significantly, at the upper exposure concentrations tested (160 μ M). The evaluation of the Cu storage within the spores in the form of Cu nanoparticles was attempted also using a combination of single particle-ICP-MS (sp-ICP-MS) and transmission electron microscopy (TEM). These techniques allowed to confirm, for the first time, the presence of endogenous Cu nanoparticles inside *S. coelicolor* spores which seemed to modulate secondary metabolism, increase actinorhodin production and prevent for Cu toxicity.

(ATOM-05.5) Characterization of Elemental and Ligated Cobalt in Vitamin B12 using the Liquid Sampling-Atmospheric Pressure Glow Discharge Microplasma

Cameron J. Stouffer¹, Sarah K. Wysor¹, Joseph V. Goodwin¹, R. Kenneth Marcus¹;

¹*Clemson University*

Intact VB12, and cobalt's effect on its structure, comprehensively determined using ESI- and LS-APGD-MS

Vitamin B12 (VB12), also known as cobalamin, is a cobalt-complexed, water-soluble vitamin essential to several homeostasis processes, including red blood cell formation, cell metabolism, and DNA production. VB12 is a common additive in cell culture media (CCM), where it is used to support cell growth in bioprocess applications. Cobalt plays a role in protein glycosylation in CCM, but in excess, it can cause reactions with the oxygen species, which results in the damage of the cells in the CCM. Recent studies have focused on characterizing the speciation of metal ions in CCM through reversed-phase separations. In CCM, cobalt was found primarily as a free ion, leading to the speculation that VB12 may break down in CCM, and the cobalt core may detach from the molecule, acting as a free ion. As cobalt is present as a free ion, this may suggest that it has a purpose in CCM functionality that was not previously known. Determining the cobalt complexation in CCM will improve the formulation knowledge and the effects that the contained cobalt has on the functionality of VB12 in CCM, benefitting both the media manufacturer and biopharmaceutical producers. Here, a

liquid sampling-atmospheric pressure glow discharge (LS-APGD) MS ion source (acting as a combined atomic and molecular (CAM) ionization source) and an electrospray ionization (ESI) source are employed to determine the speciation of cobalt in VB12 - and cobalt chloride-spiked samples comprehensively. The LS-APGD ion source can ionize polar and nonpolar compounds, as well as elemental species, which, when coupled with mass spectrometric detection, can provide a comprehensive characterization of the cobalt species in media. During this investigation, VB12 was observed to fragment into parts of the fully intact molecule, as well as rearrange into combined fragments, revealing the functional groups of VB12 that may be responsible for its functionality and efficacy. VB12, as well as cobalt-chloride spiked samples, were seen to form cobalt-associated fragments, suggesting an unknown functionality of cobalt in VB12 activity.

22AWD04: ANACHEM Award Symposium Honoring Joseph Loo

Chair: Joseph Loo

Co-Chair: Rachel Ogorzalek Loo

(AWD-04.1) Multidimensional Mass Spectrometry of Advanced Materials

Chrys Wesdemiotis¹, Chrys Wesdemiotis¹; ¹*University of Akron*

Progress in science and engineering relies increasingly on synthetic macromolecules with well-defined structures, optimized to perform specific biomedical, technological, consumer, and environmental applications. Mass Spectrometry (MS) using matrix-assisted laser desorption/ionization (MALDI) and/or electrospray ionization (ESI) provides a powerful and versatile tool for the molecular characterization of such compounds as well as for imaging the molecular composition and defects of solid polymer surfaces. The dispersive nature, high mass resolution, and unparalleled sensitivity of MS permit the conclusive and confident elucidation of molecular weight (MW), functionality, and end group distributions and are particularly suitable for monitoring reactions that change the mass. This information is imperative for both structural elucidation as well as the determination of polymerization mechanisms. Complex mixtures and multicomponent blends can be simplified with multidimensional approaches, involving in-line separation by polarity, using liquid chromatography (LC), and/or by ion size and shape, using ion mobility (IM) spectrometry. Meanwhile, large macromolecules and crosslinked networks can be made analyzable by mild thermal degradation that predictably breaks weak bonds while preserving connectivity and functionality. Conversely, mass spectrometry imaging (MSI) techniques with solvent-free MALDI can be utilized to determine the molecular composition and defects in the top molecular layer of synthetic polymer surfaces (<2 nm), which can differ from the composition of the bulk due to segregation or environmental factors. In all cases, additional insight about primary structure, architecture, and topology can be gained through tandem mass spectrometry (MS/MS) fragmentation. Our group has utilized such multidimensional approaches to elucidate the microstructures of a broad range of polymeric materials that are widely used but challenging to characterize at the molecular level by other analytical

techniques. The examples to be presented include variously shaped (co)polymers, complex conjugate blends, crosslinked materials, and solid surfaces.

(AWD-04.2) **Coupling Accelerated Droplet Chemistry with LC-MS for Saccharide Analysis**

Abraham Badu-Tawiah¹, Enoch Amoah, Derik Heiss; ¹*The Ohio State University*

Recent developments have transformed mass spectrometry (MS) into an all-purpose technique for chemical analysis, but quantitative MS has remained challenging. Often, ion intensities measured in the gas-phase do not reflect analyte concentration in solution. The current presentation will discuss an approach that fundamentally advances analytical MS by developing methods that combine ion generation and reaction into a single step. The underlying scientific premise is based on the following hypothesis: since most mechanisms related to ion suppression in electrospray ionization (ESI) MS occur in the charged droplet environment, it is necessary to develop methods that overcome ion suppression during the stages of the droplet formation (not before, not after). The accelerated reaction rates, typical under the droplet condition, make possible rapid and efficient chemical detection, which we apply for on-line modification of saccharides to improve their ionization efficiency and isomer differentiation. Saccharides play critical roles in many biological processes. The structure of saccharides is challenging to elucidate because of the diversity of the constituent monosaccharides, anomeric configuration, and glycosidic linkages. By coupling accelerated droplet chemistry with liquid chromatography MS, we have achieved femtomole limits of detection of (oligo)saccharides in biological fluids (e.g., urine and plasma). The use of phenylboronic acid reactions and halide adduction in droplet chemistry, post-column, also provided a general platform for analysis of saccharide isomers. For example, through tandem MS, Both reactions offered a facile approach to differentiate five positional isomers of sucrose, including trehalulose, turanose, maltulose, leucrose, and palatinose. Aside from biofluid analysis, the method is applied to analyze and distinguish isomeric species present in different honey samples.

(AWD-04.3) **Lysine Acylation is Linked with Metabolism in Syntrophic Communities**

Rachel Ogorzalek Loo¹, Janine Fu, Robert Gunsalus, Michael McInerney, Joseph A. Loo¹; ¹*University of California, Los Angeles*

The post-translational modification (PTM) N- ϵ -lysine acylation regulates diverse biological mechanisms intersecting metabolism. The intrinsically reactive metabolites, reactive acyl-CoA species (RACS), drive non-enzymatic acyl modification. Different metabolic pathways generate a variety of RACS that, in turn, produce a diverse array of acyl-modifications. Characterizing these PTMs comprehensively is important to understand their impact on metabolic regulation. Although advanced MS maps acetyl proteomes, capturing the acylome without bias has been challenging. Without using PTM-specific enrichment, we identified 6 types of acyl-PTMs in syntrophic bacteria.

The link between protein acylation and metabolism was investigated by altering syntrophic consortia's carbon sources and characterizing the acyl-proteomes. We observed acetyl, butyryl, 3-hydroxybutyryl, crotonyl, valeryl, and hexanoyl modifications, (two of which are

novel PTMs), totaling over 400 modified sites on 200 proteins. Considering a large number of possible acylations dramatically expands the proteomic search space, greatly increasing potential misidentifications from isomeric and/or isobaric combinations. Consequently, we utilized unique acyl-modification specific immonium ions for further validation.

A majority of the modified proteins contribute to metabolism and those in one particular pathway, butyrate degradation, are heavily modified, presenting over 150 unique, acylated peptides. *Interestingly, the abundance of these enzymes varies little across growth conditions, suggesting that metabolism must be regulated directly by the acylations.* The number and type of modifications mirror the buildup of RACS at known metabolic bottlenecks in the degradation pathway. Valeryl-lysine and hexanyl-lysine modifications are only observed when cells are grown on longer fatty acid substrates, illustrating how these modifications derive from the environment.

The necessity of employing MS/MS marker ions for validating longer chain acyl-PTMs isobaric with other modifications and residues is demonstrated. Many of the modified sites show extreme heterogeneity in the types of acylation observed and diagnostic marker ions are essential to ascribe them confidently. Targeted PRM experiments reveal that acyl-peptide variant abundances change significantly according to carbon source, suggesting an intra-protein crosstalk between PTMs. For instance, we observe that butyryl-lysine containing PTM combinations are upregulated in butyrate grown cells. These findings illustrate the value in characterizing the acylome comprehensively and demonstrate the intricacies of acyl-PTMs in metabolism.

(AWD-04.4) Next-Generation Protein Stability Measurements in the Absence of Bulk Solvent

Brandon Ruotolo¹, Brandon Ruotolo¹; ¹*University of Michigan*

Medicines of the future will rely heavily upon our ability to quickly assess the structures and stabilities of such complex macromolecular machines, as well as the influence of large libraries of conformationally-selective small molecule binders and protein-based biotherapeutics. Such endeavours are nearly insurmountable with current tools. In this presentation, I discuss recent developments surrounding collision induced unfolding (CIU) methods that aim to bridge this technology gap. CIU uses ion mobility-mass spectrometry (IM-MS) to measure the stability and unfolding pathways of gas-phase proteins, without the need for covalent labels or tagging, and consuming 10-100 times less sample than almost any other label-free technology. Recent developments in high-throughput CIU screening methods, their ability to track alterations in target structure over a wide array of proteoforms, and software developments that seek to enhance CIU information content, will be discussed.

(AWD-04.5) From Protein Biochemist to Protein Mass Spectrometrists

Kenneth D. Greis¹; ¹*University of Cincinnati*

For protein biochemists some of the critical breakthroughs in mass spectrometry technologies came in the late 1980s and early 1990's with the ability to ionize, detect, fragment and sequence proteins and peptides from liquid interfaces coming to the forefront

of technology. At the same time advances in user friendly mass spectrometers, computer interfaces to control the instrumentation, and computational tools for data analysis led to an explosion of biological applications. Today's awardee, Joseph A. Loo, PhD was in the thick of this transition as a pioneer in the ionization, analysis, fragmentation, and characterization of proteins via mass spectrometry (Loo JA, *et al.*, *Rapid Comm. Mass Spectrom.* 1988, 2:207; Loo JA, *et al.*, *Anal. Biochem.* 1989, 179:404; Loo JA, *et al.*, *Science* 1990, 48:201). His work, along with others, influenced a whole generation of biologists and biochemists to adopt mass spectrometry as a primary tool to understand biological function. This presentation will focus on how a protein biochemist became an early adopter of mass spectrometry to address practical questions across a wide scope of biological processes and diseases. As an example, the presentation will highlight recent comparative phosphoproteomics studies to elucidate therapeutic targets for oncogenic mutations in granulocyte colony stimulating receptor in patients with Severe Congenital Neutropenia.

22BIM02: BioPhotonics Technologies Fighting Infections at the Point of Care

Chair: Ute Neugebauer

Co-Chair: Jürgen Popp

(BIM-02.1) Automated Raman Spectroscopic Pathogen and AMR Detection from Research Lab to Diagnostic Solutions

Markus Lankers¹; ¹*mibic GmbH & Co. KG*

Rapid microbiological methods and point of care diagnostics are dominated by molecular biological DNA/RNA based methods or antibody assays combined with comparatively simple optical methods for detection such as UV/VIS or fluorescence.

Modern spectroscopic methods can answer complex diagnostic and microbiological questions. Raman spectroscopic methods can identify individual bacteria without any growth steps. In addition, further characteristics of the germs such as a phenotypic characterization of antibiotic resistances are possible.

The GRAMRAY technique presented here enables the automatic detection of bacteria, by combining darkfield and fluorescence microscopy and identification of the detected bacteria within a few minutes. The technique can further realize dead/live differentiation and Antimicrobial Resistance (AMR) testing if required. After conversion to an easy-to-use instrument and sample preparation development, various applications could be realized together with customers from the food industry, industrial microbiology, diagnostics and pharmaceutical industry, whose benefits are mainly based on the speed of the technique. LOD values of 100 CFU for gene therapy samples and 1000 CFU for fruit juice concentrates are achieved. In addition to these applications, the key factors that are critical to the success of the technique will be highlighted:

- Integration into the existing analytical environment
- Robustness of the method
- Simplicity of sample preparation
- Price/price of the sample

Especially the last 2 points are crucial for the application as POC diagnostics and provide the basis for the further successful development of the GRAMRAY technique.

(BIM-02.2) SERS Combined with Chemometric Analysis for Detection and Identification of Bacteria.

Agnieszka Kamińska¹, Agnieszka Kamińska¹, Sylwia Berus, Krzysztof Niciński, Evelin Witkowska, Monika Adamczyk-Popławska, Beata Młynarczyk-Bonikowska,, Tomasz Szymborski; ¹*Polish Academy of Sciences*

The present work demonstrates that surface-enhanced Raman scattering (SERS) coupled with biochemical methods and chemometric analysis (PCA, PLS-DA, an SIMCA) is a reliable and fast method for detection and identification of pathogenic bacteria from clinical and environmental samples. The proposed SERS-based method for bacteria identification challenges the standard biochemical methods in terms of simplicity, specificity and rapidity. The direct SERS analysis of bacteria (even single bacteria cell) is performed directly from SERS-active nanostructures incorporated into a microfluidic module. The developed SERS-active nanostructures are based on the femtosecond laser induced silicon and exhibit very strong enhancement factor (up to 10^8), high stability and reproducibility, which satisfies all spectroscopic features required for the direct, intrinsic format of the spectroscopic analysis in the developed biosensors. The recorded SERS data of bacteria are categorized (assigned to particular bacterial species) using data analysis software based on established SERS database of bacteria. The long time of incubation of bacteria is eliminated and the total analysis including numerical analysis of recorded SERS data will not exceed 15 minutes. Additionally, the proposed FORMI device can be introduced to International Organization for Standardization (ISO) standards for bacteria identification, to avoid the time-consuming methods routinely used in laboratories. Presented approach opens a new path in microbiological diagnostics for sensitive, simple, quick, and on-site detection of pathogenic bacteria including environmental and clinical microbiology (hospitals, health centre), food industry and environmental protection.

(BIM-02.3) Label-free, Raman-Based Analysis of Leukocytes for Rapid Characterization of Immune Response to Infection

Ute Neugebauer¹, Natalie Arend¹, Anuradha Ramoji¹, Daniel Thomas-Rüddel², Oleg Ryabchykov¹, Aikaterina Pistiki¹, Michael Kiehntopf², Frank Bloos², Thomas W. Bocklitz³, Iwan Schie⁴, Michael Bauer², Juergen Popp⁵; ¹*Leibniz Institute of Photonic Technology*, ²*Jena University Hospital*, ³*Leibniz Institute of Photonics Technology*, ⁴*Leibniz Institute of Photonic Technology*, ⁵*Friedrich-Schiller University*

Raman spectroscopy is a powerful biophotonic technology that can deliver in-depth information about biological specimen in short analysis time in a labelling-free and non-destructive manner. Recent technological advances made it possible to collect spectral information from individual cells in high-throughput [1], paving the way for potential diagnostic applications.

In this contribution, we will present how Raman spectroscopy is used to characterize immune cells, differentiate their phenotype, and follow their response to pathogens and pathogen-associated molecular patterns (PAMPS). We also outline their potential impact for the diagnostics of infection and identification of dysregulated immune responses as encountered in sepsis.

The counts of leukocyte subtypes in peripheral blood gives valuable information about patient's health status and its assessment is therefore part of the daily lab routine. Using high-throughput Raman spectroscopy, differential blood counts of the major leukocyte subtypes can be obtained in a direct, label-free and non-destructive manner showing good agreement to clinical values [1]. In addition, specific phenotype information can be extracted from the Raman spectra of the immune cells, e.g., differentiation of activated phenotypes after *in-vitro* or *in-vivo* stimulation.

Time-resolved analysis of spectral changes in THP-1 monocytes after *in-vitro* stimulation with the PAMP lipopolysaccharide revealed metabolic changes confirmed by gene transcription and cytokine analysis [2]. Specific activation states of primary isolated human leukocytes after *in-vitro* stimulation with intact bacterial and fungal pathogens could be differentiated [3, 4] and the characteristic Raman spectroscopic fingerprints be used to predict the activation agent (bacterial vs. fungal).

Sample preparation was optimized to require only minimal amounts of blood to perform leukocyte analysis of patient's samples. In a first translational trial, the added value of the Raman spectroscopic leukocyte profile was demonstrated for the identification of infection and sepsis [5].

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(BIM-02.5) Asymptomatic Malaria Detection using Microfluidic Paper Device Capable of On-Chip Mass Spectrometry

Abraham Badu-Tawiah¹, Suji Lee¹, Ayesha Seth¹, Girish muralikrishnan¹; ¹*The Ohio State University*

The first application of miniature mass spectrometry in malaria diagnosis using protein biomarker detection

Surveillance is highlighted as a worldwide strategy for disease prevention. An effective surveillance plan targets both symptomatic and asymptomatic patients. In this presentation, an innovative diagnostic platform will be described that combines immunoassays performed in paper substrates with on-chip analysis by a miniature mass spectrometer. The signal transduction strategy utilizes a dendrimer-based amplification method that allows mass spectrometry (MS) ion signal to be enhanced. This allows for ultra-sensitive detection that is necessary for asymptomatic malaria diagnosis. Asymptomatic malaria occurs in approximately 80% of the population in sub-Saharan Africa. Based on its high sensitivity, our paper-based MS platform provides malaria care in three unique levels, including (1) prompt diagnosis of symptomatic patients, (2) remote sampling targeting asymptomatic patients, followed sensitive mass spectrometry analysis at a later time, and (3) field study where the whole community can be screened, and diagnostic results gathered in real-time. Result will be presented that include a recent surveillance performed in two (rural and urban) communities in Ghana, West Africa. These features are possible because sample collection and analysis can be accomplished in two independent steps and the paper device can be stored at room temperature with no need for cold storage. Using the dendrimer-based signal transduction, we achieved 4.5 pM (< 10 parasites per μ L blood) sensitivity for *Plasmodium falciparum* malaria using histidine-rich protein 2 (*PfHRP2*) antigen as biomarker. This presentation will discuss performance for miniature and bench-top mass spectrometers using clinical samples collected from Ghana. We will validate results using well-known sensitive methods such as polymerase chain reaction, enzyme-linked immunoassay, and the gold standard light microscopy. Based on these comparisons, the selectivity and specificity the new paper-based MS method will be calculated and reported. This work represents that first report for using miniature mass spectrometer to analyze large protein biomarkers from clinical whole blood samples.

22FORENS01: Nuclear Forensics

Chair: Robert Lascola

(FORENS-01.1) Microscopy and Spectroscopy of Actinide Dioxide Aging as a Function of Temperature and Relative Humidity

Amy E. Hixon¹, Meena Said², Samuel Perry¹, Savannah Benjamin¹; ¹*University of Notre Dame*, ²*Lawrence Livermore National Laboratory*

The characterization of nuclear materials under controlled conditions promotes a better understanding of their chemistry and provides insight into their stability. Of particular importance is identifying how environmental and storage conditions affect both bulk and sub-bulk scale chemical and physical properties. Such data may provide insight for nuclear forensic applications and can help address fundamental safety and storage concerns. In this work, thorium dioxide (ThO₂), uranium dioxide (UO₂), and plutonium dioxide (PuO₂) aging was studied as a function of time, temperature, and relative humidity (RH) while simultaneously observing the effects of fluoropolymer containment. Morphological changes were monitored using scanning electron microscopy and interpreted using a previously-published lexicon of descriptive imaging terminology. Changes in phase were observed using X-ray diffraction measured on a single crystal X-ray diffractometer to permit sub-bulk scale (< 10 mg) analysis. Ancillary techniques, including energy-dispersive X-ray, infrared, Raman, and X-ray photoelectron spectroscopy, were used to further probe and interpret aging effects. ThO₂ materials exhibited no change in macroscopic appearance and microstructure. Over time, UO₂ transitioned to a mixed phase assemblage. Among the alteration was ammonium uranate, a common fuel cycle compound, which was identified from its morphological and spectral characteristics. PuO₂ underwent transitions to mixed plutonium dioxide/plutonium fluoride hydrate and mixed plutonium dioxide/ammonium plutonium fluoride phases under ambient temperature at 81% RH and 45 °C at 81% RH, respectively. These results highlight important stability differences between actinide oxides and underscore the need for further research targeted at understanding the fundamental chemical and physical properties of actinide compounds near ambient temperatures and humidities.

(FORENS-01.2) Laser Fluorescence Spectroscopy and Multivariate Chemometrics for the Quantification of Uranium(VI), Samarium, Nitric Acid, and Temperature

Luke Sadergaski¹, Luke Sadergaski¹, Hunter B. Andrews¹; ¹*Oak Ridge National Laboratory*

Laser induced fluorescence spectroscopy (LIFS) and multivariate chemometrics has been used to simultaneously determine uranium(VI) (1–100 ppm), samarium (0–200 ppm), and nitric acid (0.1–4 M) with varying temperature (20–45°C). LIFS applications range from fundamental lab-scale studies to real-time process monitoring at industrial levels such as nuclear reprocessing applications, provided the phenomena affecting the fluorescence spectrum are accounted for (e.g., absorption, quenching, complexation). Results obtained on synthetic samples selected by D-optimal experimental designs indicate that stacked regression methods can be used to determine uranium(VI) concentrations in the submilligram-per-liter range directly in nitric acid without measuring luminescence lifetimes or standard addition. This work also demonstrates the ability to quantify fluorescent fission products (e.g., Sm³⁺) in the milligram-per-liter range using a 405 nm excitation wavelength. This framework reinforces the applicability of LIFS for online analysis in nuclear fuel cycle applications.

(FORENS-01.3) Simultaneous Determinations of Uranium and Plutonium Utilizing Ultra-high Mass Resolution: The Liquid Sampling Atmospheric Pressure Glow Discharge/Orbitrap Coupling

Joseph V. Goodwin¹, Benjamin T. Manard², Brian Ticknor², Paula Cable-Dunlap², R. Kenneth Marcus¹; ¹*Clemson University*, ²*Oak Ridge National Laboratory*

Reported is the simultaneous detection of plutonium and isotope ratio measurement of uranium without pretreatment.

Coupling the liquid sampling atmospheric pressure glow discharge (LS-APGD) ionization source with an Orbitrap mass spectrometer has proven to be a powerful and lower operational overhead analytical solution for the nuclear regulatory community. The LS-APGD/Orbitrap coupling has proven to be a versatile technique for uranium isotope ratio determinations, with recent studies reporting the use of the LS-APGD/Orbitrap combination to analyze plutonium. The measurement of uranium (U) and/or plutonium (Pu) is essential for nuclear safeguards. Generally, the measurement of Pu requires a separation from the U for an accurate determination due to the molecular interferences that could be present (i.e., ²³⁸UH and ²³⁹Pu). To further expand the capabilities of the LS-APGD/Orbitrap coupling in this regard, the simultaneous determination of plutonium and uranium isotope ratio measurements (without prior sample treatment) is presented. The ultra-high mass resolution offered by the Orbitrap mass spectrometer could eliminate the complex sample manipulations commonly employed to remove isobaric interferences with traditional uranium isotope ratio determination techniques, such as thermal ionization mass spectrometry (TIMS) or inductively coupled plasma-mass spectrometry (ICP-MS). In addition, the LS-APGD ionization source operates with significantly reduced solution (< 100 $\mu\text{L min}^{-1}$) and gas flow rates (< 0.7 L min^{-1}) compared to ICP-MS instrumentation, which, when combined with a benchtop Orbitrap mass spectrometer, allows for a forward-deployable platform for uranium isotope ratio analysis with simultaneous plutonium determinations. A Spectroswiss FTMS Booster X2 external data acquisition system was used in combination with the LS-APGD/Orbitrap coupling, resulting in increased resolution from extended transient lengths and increased sensitivity through its ability to process data without the automatic noise removal step, which is typical with the “reduced profile mode mass spectra” provided by the Orbitraps standard data acquisition system. Parametric optimization for the dual-electrode LS-APGD ionization source included solution flow rate, sheath gas flow rate, inter-electrode gap distance, and current to find conditions that resulted in the highest sensitivity for plutonium while also maximizing the accuracy of uranium isotope ratio determinations. In addition, optimal ranges for in-source collision-induced (CID) and higher-energy collisional dissociation (HCD) for the uranium and plutonium samples were determined.

(FORENS-01.4) Reaction Dynamics Of The Hydrolysis Molybdenum Hexafluoride By Cryogenic Layering On A Diamond Substrate

Abigail M. Waldron¹, K. Alicia Strange Fessler¹, Patrick O'Rourke¹, Louis McNamara¹, Michael Thomas¹; ¹*Savannah River National Laboratory*

This work presents the first results on a novel method to study metal hexafluoride hydrolysis.

Depleted uranium hexafluoride (UF₆) is a stockpiled byproduct of the nuclear fuel cycle. UF₆ will react readily with atmospheric humidity, but the mechanism by which this reaction proceeds is poorly understood. If released into the atmosphere, UF₆ reacts with water to form chemically toxic and radioactive reaction products, uranyl fluoride (UO₂F₂) — a soluble solid — and HF gas with a stoichiometric ratio of UF₆(g)+2H₂O(g) → UO₂F₂(s) + 4HF(g). Previous UO₂F₂ particulate research has shown the end state of the chemical reaction to be dependent on the amount of water present in the atmosphere. In this work, we have used molybdenum hexafluoride as a non-radioactive substitute for uranium to study the hydrolysis of metal hexafluorides. As part of developing a method for studying the kinetics of a spontaneous gas phase reaction, molybdenum hexafluoride gas and air were sequentially layered on a diamond substrate kept at liquid nitrogen temperature (around -193 °C). This was achieved using a custom designed cryogenic cell with a copper cold finger and held at vacuum. Reaction progress was monitored via FTIR to ensure no reaction was occurring in the frozen state, and then over several hours while allowing the substrate to warm up to room temperature. The recorded spectra showed noticeable differences between MoF₆ hydrolysis products and known UF₆ products. Products were characterized via UV/Vis, x-ray diffraction, x-ray fluorescence, scanning electron microscopy, and FTIR.

(FORENS-01.5) Simultaneous DSC–FTIR Reflectance Spectroscopy of the Insensitive High Explosive Triaminotrinitrobenzene (TATB) undergoing Thermal Degradation
Greg L. Klunder¹, Malik Oliver¹, Batikan Koroglu¹, Keith Coffee¹, Adele Panasci-Nott¹, Joseph Van horn¹, Evan Kahl¹, Taylor Miller¹, Alan Burnham¹, John Reynolds¹; ¹LLNL
Provides new chemical data of the thermal degradation of TATB.

Triaminotrinitrobenzene (TATB) is a popular high explosive for several military and non-military uses due to insensitivity to temperature, shock, and pressure. How this insensitivity changes with exposure of TATB to high temperatures is of concern and because chemical information is incomplete. Several proposed kinetic models for thermal degradation do not accurately predict what is observed with differential scanning calorimetry (DSC) which shows two exotherms. To evaluate the chemical changes of the solid material in real time, a DSC has been interfaced with an FTIR microscope to measure the reflectance spectra thru a pinhole in the lid of the DSC crucible. Most of the DSC analyses were performed with a 10°C/min temperature ramp to 330°C where it was held for extended times. Shifts in the symmetric and asymmetric NH vibrational bands are observed during the initial heating indicating a weakening of the intramolecular hydrogen bonding between the NH₂ and NO₂

groups. Spectral changes under isothermal conditions indicate an early loss of NO₂ groups, consistent with other observations from separate studies. This project brings together several analytical techniques to help elucidate the thermal degradation mechanism of TATB and the sensitivity of the resulting decomposition products. This presentation will cover the experimental considerations for DSC-FTIR, nuances of the spectral interpretations, chemical composition with supplemental LC-MS analysis including evaluations using isotopically labeled TATB.

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-ABST-#####.

22IR02: NanoIR in Life Science and Biology

Chair: Francesco Simone Ruggeri

(IR-02.1) Nanoscale Structural Analysis of a Lipid-Driven Aggregation of Insulin

Dmitry Kurouski¹, Dmitry Kurouski¹; ¹*Texas A&M University*

Abrupt aggregation of misfolded proteins is a hallmark of a large number of severe pathologies, including diabetes types 1 and 2, Alzheimer, and Parkinson diseases. A growing body of evidence suggests that lipids can uniquely change rates of amyloid-associated proteins as well as modify the structure of formed oligomers and fibrils. In this talk, I will demonstrate the advantage of atomic force microscopy infrared (AFM-IR) spectroscopy, also known as nano-IR spectroscopy, in nanoscale analysis of secondary structure of individual insulin oligomers, protofilaments, and fibrils grown in the presence of phospholipids. Our findings show that AFM-IR spectra of insulin oligomers have strong signals of C-H and PO²⁻ vibrations, which points on the presence of lipids in the oligomer structure. Furthermore, substantial shifts in lipid vibrations in AFM-IR spectra of the oligomers relative to the corresponding bands of pure lipids have been observed. This points on strong interactions between a lipid and a protein that are developed at the stage of the oligomer formation.

(IR-02.2) Peak Force Infrared Microscopy for Label-free Chemical Imaging of Biological Structures

Xiaoji Xu¹; ¹*Lehigh University*

Abbe's diffraction limit means that traditional infrared microscopy has a spatial resolution on the micrometer level, much larger than the characteristic scales of many biological samples, from proteins to cells. In this presentation, I will describe our recent applications of peak force infrared (PFIR) microscopy, a new type of nano-IR technique with multimodal characterization at <10 nm spatial resolution. PFIR microscopy utilizes temporal domain mechanical detection of the tip-enhanced infrared photothermal response

of the sample with a nanoscopic atomic force microscope (AFM), operated in the peak force tapping mode. The method simultaneously yields chemical and mechanical images of the sample, and in our recent development, simultaneously with surface potential mapping. We have demonstrated the multimodal imaging capability of PFIR on a wide range of biological objects, from amyloid fibrils to cellular structures. We recently upgraded the PFIR microscopy to operate within the liquid phase to form the method of liquid phase peak force infrared (LiPFIR) microscopy. It opens the route to in situ biological imaging at the liquid/solid interface. Finally, we demonstrate the aqueous phase imaging of structured polymers, proteins, and cells, as well as the feasibility of using LiPFIR to track the click chemistry reactions.

(IR-02.3) **Nanoscale Bio-Spectroscopy using Multivariate Data Analysis**

Georg Ramer¹, Bernhard Lendl¹, A. Catarina V.D dos Santos¹; ¹TU Wien

In this work we focus on applying multivariate methods to scanning probe based photothermal nearfield mid-IR spectroscopy (usually referred to as AFM-IR). AFM-IR spectra are often described as resembling bulk absorption spectra. However, in contrast to bulk absorption spectra, AFM-IR spectra do not follow Beer's law and thus their signal does not depend linearly on the analyte concentration. Furthermore, at nanoscale spatial resolution lateral sample drift is noticeably affecting sample positioning. The optical non-linearity can be addressed by sample preparation and careful choice of sampling parameters.

Thermal drift is a bit more challenging to solve in practice. Instead of trying to minimize drift beyond what is typically achievable in a conventional AFM-IR setup, we have developed several strategies (measurement procedures and software routines) that allow us to acquire high resolution (both high spatial resolution and high pixel resolution) hyperspectral images in presence of thermal drift. These strategies allow us to apply multivariate methods to AFM-IR hyperspectral images of micro-organisms, vesicles and other chemically complex nanoscale structures without losing information about measurement position and artifacts due to offsets between images.

(IR-02.4) **Application of Nano-FTIR Technology in Amyloid- β (A β) Research: A Revolutionary Tool in Disease Diagnosis**

Tobias Gokus¹, Suman Paul¹, Artem Danilov¹; ¹Attocube Systems AG

Nanoscale infrared imaging and spectroscopy for studying drug-induced structural changes of amyloids

Amyloid- β (A β) is a class of misfolded proteins responsible for neurodegeneration posing severe threats to human health. It is a short peptide sequence, swiftly aggregates, albeit heterogeneously making it difficult to distinguish based on morphology alone. Any interaction with promoters or inhibitors/drugs modifies the size and shape of these aggregates,

adding extra hurdles to A β research. It is well known that any simple conventional imaging such as offered by electron microscopy (EM) and/or atomic force microscopy (AFM) lacks chemical sensitivity and therefore not sufficient to discriminate the various structures in the aggregates. However, due to its high spatial resolution of 10 nm and its capability to probe the local chemical and structural properties, the nano-FTIR technology is inherently designed to address this kind of challenges [1].

Scattering-type Scanning Near-field Optical Microscopy (s-SNOM) is the most popular choice for nano-FTIR devices. An infrared broadband radiation is confined to the apex of a metallic AFM tip (tapping-mode) by a parabolic mirror which also redirects the tip-scattered light to the detector where nanoscale optical absorption and reflection properties of the sample below the tip are analyzed. A Michelson-type interferometer is suitably placed in the device to perform a Fourier-Transform Infrared Spectroscopy on a nano-spot size [2-3].

Here, we report the use of s-SNOM technology on an amorphous A β sample (A β 40). A wide range of structures (as thin as 2 nm and as large as few hundred nm) were easily discriminated based on the spectral positions of the amide I band of the protein backbone [4].

References:

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2. Keilmann, F. and R. Hillenbrand, *Near-field microscopy by elastic light scattering from a tip*. Philos Trans A Math Phys Eng Sci, 2004. **362**(1817): p. 787-805.
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22PMA05: Industrial Applications of Vibrational Spectroscopy

Chair: Patrick Wray

Co-Chair: James Kimber

(PMA-05.1) The Importance of Spectral Pre-processing for On-line Process Analysis Using Vibrational Spectroscopy

Alison Nordon¹; ¹*University of Strathclyde*

Vibrational spectroscopic techniques are being used increasingly for on-line process monitoring in the chemical-using industries. Chemical processes often contain multiple components, and therefore, multivariate analysis is usually required to extract the information of interest from spectra. A number of factors contribute to vibrational spectra including the chemical composition, temperature, and physical properties such as particle size. Hence, spectral pre-processing is required to remove the effects of properties that are undesirable while retaining the information of interest. In this presentation, the importance of spectral pre-processing for on-line process analysis using vibrational spectroscopy will be illustrated using a number of examples.

In the first example, the use of attenuated total reflectance (ATR) mid infrared (MIR) spectroscopy for the monitoring of solute concentration during cooling crystallisation is presented. The effects of temperature on the spectra were removed using loading space standardisation (LSS). It was shown that more accurate prediction of solute concentration was obtained using spectra pre-processed using LSS than with a global partial least squares (PLS) model. In the second example, the use of Raman spectroscopy is described for quantitative analysis of powder blends exhibiting variations in physical properties. Construction of accurate PLS calibration models requires use of pre-processing algorithms to remove the effects of light scattering from spectra. In this example, a novel dual calibration algorithm was used, which gave more accurate and robust prediction of extent of conversion. In the final example, near infrared (NIR) spectrometry is used for on-line monitoring the final stage of a fermentation process. A calibration transfer algorithm was used to maintain the performance of a PLS calibration model when the experimental conditions were changed.

These examples illustrate that vibrational spectroscopic techniques, in conjunction with multivariate analysis, can be used for on-line monitoring of a wide range of processes in the chemical-using industries.

(PMA-05.2) Infrared Spectroscopic, Imaging and Nano-Spectroscopic Analysis of Cells for Drug Development

Andrew Chan¹; ¹*King's College London*

Fourier transform infrared spectroscopy is a chemically specific (label-free) analytical method that has shown to be useful in the study of cells in order to understand drug-cell interactions. It has been used to study a wide range of cellular events and diseases, e.g., distinguishing the different stages of the cell cycle, cell death, diagnosis of diseases including cancers. Recently, our group has focused on the study of cell (live and fixed) exposed in drugs in order to reveal the cell-drug interactions at the molecular level. We have demonstrated that the method is useful in the quantification of drugs in living cells, revealing the mode of

action of drugs, understanding the biochemical changes inside cells as a result of the presence of drugs.^[1] We have also extended our study to reveal the effect of anti-diabetic drugs on live diabetic model cells showing the restoration of the biochemical composition inside cells.^[1c] To achieve these, we have developed a live-cell FTIR measurement approach to ensure high-quality and highly reproducible spectra can be collected. We have developed novel solid immersion method using two ZnS hemispherical lenses^[2] and collected resonance enhanced AFM nano-FTIR at the Diamond Light Source B22 beamline^[3] to increase the spatial resolution of FTIR imaging of live cells and fixed cells at a subcellular and sub-micron levels, respectively.

In this presentation, we will be presenting a summary of these recent developments to demonstrate that FTIR can be a powerful tool for understanding drug-cells interactions to aid the development of new and better medicine.

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[2] K. L. A. Chan et al, *Bioanal. Chem.* 2018, 410, 6477-6487.

[3] K. L. A. Chan et al, *Anal. Chem.* 2020, 92, 8097-8107.

(PMA-05.3) **Applications of Spectroscopic Imaging and PAT to 3D Printed Formulations**

*Zoë Whalley*¹, *Patrick Wray*², *Tom Mills*¹, *Richard Greenwood*¹; ¹*The University of Birmingham*, ²*Bristol Myers Squibb*

3D printing of pharmaceuticals is an emerging field, which will have many exciting applications in the sphere of personalised medicine. The inherent advantages are that it offers manufacturing flexibility and platform versatility. The first FDA approved 3D-printed drug has been on the market since 2015; hence there is a great deal of innovation and research in this area, to further increase our understanding of the potential applications of this technique.

This research focuses on a type of 3D Printing known as Fused Deposition Modelling (FDM). A filament is produced by combining a polymer with an active pharmaceutical ingredient in a hot melt extruder (HME). The printer then melts the filament and deposits it onto a print bed layer by layer to form the 3D printed tablet. Complex, novel structures are possible using this 3D printing technique, which would be otherwise unobtainable with traditional compaction methods. The complex shapes can be used to accurately control the drug release from the 3D matrix.

Dispersing the active drug in a polymer matrix via HME can help to retain the drug in its amorphous form. The amorphous form of a drug is often advantageous due to its increased solubility, and hence better bioavailability compared to the crystalline form. This is particularly beneficial when investigating drugs with a Biopharmaceutical Classification System (BCS) class of II or IV, meaning they are not readily soluble in the body.

Vibrational spectroscopy has the potential to significantly improve understanding of all aspects of these novel formulations. Process analytical technologies can be used in a non-destructive manner to monitor the manufacture and printing processes, as well as the long-term stability of the finished tablets. In this work chemical imaging is used to provide a series of global images of the dissolution of a 3D printed dose, as a function of time. This facilitated the tracking of drug distribution changes in the tablet.

(PMA-05.4) Process Analytical Technology: Applications to Batch and Flow Processes for Active Pharmaceutical Ingredient Development

Courtney Talicska¹, Howard Ward¹, Eamon O'Connell¹; ¹*Pfizer*

Process analytical technology is applied to batch and flow chemical processes for improved API development.

Process analytical technology (PAT) is revolutionizing the analysis of chemical processes throughout the pharmaceutical industry, leading to better-informed and streamlined active pharmaceutical ingredient (API) development. APIs formed in pharmaceutical processes have long relied on off-line analytical methods which, while effective in generating data, often require extended time from sample extraction to reporting of the results. Off-line analyses also require physical sampling of a chemical process, which can be hazardous to personnel and potentially be detrimental to the reaction itself. To reduce the challenges associated with off-line analyses, PAT methods can be implemented for *in situ* analysis of pharmaceutical processes. PAT provides real-time data that leads to improved process understanding and control, and reduces or eliminates the need for manual sampling, which protects both personnel and the integrity of sensitive reactions. Many PAT techniques are used in the pharmaceutical industry including: FTIR, UPLC-MS, flow NMR, Raman, and UV/Vis. These analytical methods provide data on the order of seconds to minutes, as compared to much longer times for off-line analyses. This leads to a better understanding of kinetic processes, timely reaction endpoint or steady state determination, and rigorous process control. Here, we discuss the use of PAT tools to support API development in traditional batch chemistry settings, and in flow processes as part of Pfizer's Flexible API Supply Technologies (FAST) initiative. Both qualitative and quantitative PAT approaches are described, with each method tailored to the specific chemistry being investigated. PAT is demonstrated to be beneficial for improved understanding and rapid optimization of numerous processes including hydrogenations, Grignard reactions, crystallization, high-heat

transfer reactions, and liquid-liquid extractions. The examples given here highlight the ability of PAT to reduce the need for off-line analytics, helping to accelerate development timelines for pharmaceutical APIs.

(PMA-05.5) Fluorescence Recovery after Photobleaching Based Diffusion Mapping within Heterogeneous Sample

Ziyi Cao¹, Dustin M. Harmon¹, Ruo Chen Yang¹, Minghe Li¹, Aleksandr Razumtcev¹, Garth J. Simpson¹, Lynne S. Taylor¹; ¹*Purdue University*

FT-FRAP was first implemented to map diffusion of segmented domains of arbitrary shape

The use of periodically structured illumination coupled with spatial Fourier-transform fluorescence recovery after photobleaching (FT-FRAP) was shown to support diffusion mapping for recovery of diffusivity within segmented domains of arbitrary shape. Periodic “comb-bleach” patterning of the excitation beam during photobleaching encoded spatial maps of diffusion onto harmonic peaks in the spatial Fourier transform. Diffusion manifests as simple exponential decays of the spatial harmonics in the FT-domain, improving signal to noise and simplifying mathematical analysis. Image segmentation prior to Fourier transformation was shown to support pooling for signal to noise enhancement for regions of arbitrary shape expected to exhibit constant diffusivity within a domain. Following proof-of-concept analyses based on simulations with known ground-truth maps, diffusion imaging by Fourier transform FRAP (FT-FRAP) was used to image spatially-resolved diffusion differences within phase-separated domains of model amorphous solid dispersions spin-cast thin films. Notably, FT-FRAP was able to definitively discriminate and quantify the roles of internal diffusion and exchange to higher mobility interfacial layers in mapping the recovery kinetics within thin amorphous/amorphous phase separated domains.

22RAM07: Transmission and Other Advanced Spectroscopic Sampling Methods in Pharmaceutical Analysis

Chair: Julia Griffen

(RAM-07.1) Transmission Low-Frequency Raman Spectroscopy

Motoki Inoue¹; ¹*Meiji Pharmaceutical University*

Crystalline form of active pharmaceutical ingredients (APIs) is an important aspect of optimizing drug performance. To quantify crystalline forms in solid dosage form, we attempted to use newly developed transmission low-frequency Raman spectroscopy.

In this study, we prepared two series of tablets consisting of polymorphs and cocrystals. Commercially used Raman spectrometer equipped with low frequency Raman probe (THz-Raman® probe system, Coherent Inc. CA) was used for measurement of low-frequency Raman spectra. The quantitative ability of transmission mode Raman spectroscopy was compared with backscattering mode using several chemometric techniques.

From the relationship between the contents of carbamazepine form III and partial least squares (PLS) predictions in the tablets, correlation coefficients in transmission mode ($R^2=0.98$) were found to be higher than in backscattering mode ($R^2=0.97$). The root mean square error of cross-validation (RMSECV) of the transmission mode was 3.9 compared to 4.9 for the backscattering mode. To evaluate the quantitative ability of cocrystal, design of experiments (DoE) setup was applied for the preparation of model tablets containing caffeine, glutaric acid, and caffeine-glutaric acid cocrystals. The root-mean-square error of prediction (RMSEP) was determined by comparing the actual concentration and predicted content with a calibration curve. For cocrystal-containing tablets, the quantitative ability of the transmission mode (RMSEP= 3.05) was 32.2% higher than that of the backscattering mode (RMSEP= 4.50). The coexistence of raw crystalline materials did not affect the quantitative ability for cocrystals.

These findings suggest that transmission low-frequency Raman spectroscopy is a candidate method for quantification of crystalline APIs in solid dosage forms.

(RAM-07.2) Frequency-Domain Terahertz Spectroscopy for Solid Samples in Normal Humidity Conditions with a Method for Suppressing Absorption Peaks by Water Vapor

Kei Shimura¹, Touya Ono¹, Tetsuo Sasaki², Mizuki Mohara¹, Kenji Aiko¹, Tomoaki Sakamoto³; ¹*Hitachi High-Tech Corporation*, ²*Shizuoka University*, ³*National Institute of Health Sciences*

Stable and accurate terahertz absorption spectra of hydrate forms were obtained in normal humidity conditions.

Terahertz absorption spectra of solid samples are usually obtained by locating the samples in dry conditions to avoid influence of narrow absorption peaks by water vapor in the atmosphere. This could limit its application to pharmaceutical analysis, since hydrate forms of some active pharmaceutical ingredients could be dehydrated gradually in dry conditions. A new and simple method for suppressing absorption peaks by water vapor in terahertz absorption spectra was developed and a measurement of terahertz absorption spectra of the samples in normal humidity conditions was demonstrated. In this method, only simple

mathematical operations such as subtraction, thresholding, interpolation and smoothing were applied for detected signals or calculated absorbances. By using difference in spectral line width between narrow absorption peaks by water vapor and relatively wide absorption peaks by chemicals in solid forms, absorption peaks by water vapor were effectively suppressed without affecting absorption peaks of the chemical materials in the sample. In this study, levofloxacin hemihydrate and monohydrate were used as samples. Their spectra were obtained both in a dry condition and in a normal humidity condition and also their temperature was raised to 363K to dehydrate them. Spectra obtained in the normal humidity condition were processed with the developed method. The spectra of hydrates obtained in the dry condition changed over time, but the initial spectra agreed well with spectra obtained in the normal humidity condition and processed by the developed method. Stable and reliable measurements were realized by measuring the spectra in normal humidity conditions and applying the developed method to suppress absorption peaks by water vapor. It was also shown that dehydration and hydration behaviors can be observed by the temperature-varying measurements.

(RAM-07.3) Transmission Raman as Modern Backbone of Development for Oral Solid Dosage Forms

Valentina Manici¹, Stefan Busche¹; ¹*Merck Group KGaA*

It will showcase the deployment of Transmission Raman to improve formulation and process development.

The development of tablet formulations often requires the deployment of several classical analytical pieces of equipment to gain fruitful insights into the oral solid dosage forms.

Vibrational spectroscopy techniques have been applied in several analytical development areas, such as stability testing, water content analysis, and tablet hardness determination to accelerate and gain deeper scientific insights.

This contribution will address how at Merck KGaA, Transmission Raman Spectroscopy (TRS) and Multivariate Data Analysis are deployed to support formulation development and understanding of manufacturing processes.

TRS could be a game-changer in the decision-making for Pharmaceutical Development, and not only as a valid alternative to HPLC for Content Uniformity.

Spectra from Transmission Raman spectroscopy are data-rich responses that contain chemical, as well as physical information about the samples under investigation.

Developing and applying qualitative PCA and PLS models in pipeline projects for troubleshooting purposes, exemplify how TRS and MVDA can simplify the decision-making in and for Pharmaceutical Development projects.

Examples on

TRS vs NIR Hyperspectral Imaging for double API tablets CU

Impact of Excipients and Hardness on solid oral dosage forms

Understanding the Coating process

Chemical stability

will be presented.

The generated data could be easily visualized and knowledge can be easily made available to derive the correct conclusions for multiple projects.

(RAM-07.4) The Challenge for Real Time Release of Extended-Release Formulations by Raman Spectrometry

Gregory K. Webster¹, Bharat Mankani², Sergey Mozharov², Brian Marquardt²; ¹*AbbVie*,
²*MarqMetrix*

Evaluation of using Raman for Real Time Release Testing (RTRT) of extended release formulations

The use of a surrogate application has been demonstrated using NIR with continuous manufacturing samples and is within the scope of the FDA's Real Time Release Testing (RTRT) initiative. While effective for simple formulations, this study investigates whether such a spectroscopic surrogate application can replace pharmaceutical dissolution testing for extended-release formulations.

Extended-release tablet formulations often accomplish the release rate delay through the addition of gelling agents. In this work, HPMC polymers were used to formulate extended-release niacin tablets. Because of transparency to water and numerous PAT applications, Raman spectroscopy was chosen for this investigation. Thus, in order to effectively model dissolutions profiles, the Raman technique must be able to differentiate HPMC polymers from the background and selectively distinguish between the polymers employed. Our preliminary work indicated that while Raman can effectively detect and monitor the niacin response of the tablet formulations, there is not enough unique spectral features between the HPMC polymers themselves to selectively resolve their responses. Thus, for extended-release tablet applications with continuous manufacturing, further dissolution surrogate development is needed.

22SPECIAL11: Remembering Stanley Crouch

Chair: Dana Spence

Co-Chair: F. Holler

(SPEC-11.1) A Half-Century of Working, Conducting Research, Teaching, Learning, Writing, and Laughing with Stanley Ross Crouch

F. James Holler¹; ¹*University of Kentucky*

In 1973, I was fortunate to join the research group of Professor Stan Crouch of Michigan State University Department of Chemistry. The Crouch group at that time consisted of eight veteran graduate students, and five of us incoming students joined the group that fall. In this talk, we will explore the workings of Stan's group and its evolution over the years. We shall explore various events that shaped the group and its members throughout the last five decades. We will examine how Stan's style and attitudes brought about student development and camaraderie among members.

Stan learned well from his mentors, Doug Skoog of Stanford University and Howard Malmstadt of The University of Illinois. They were both consummate teachers and researchers, but they were also excellent writers, and they inculcated this skill in Stan. Fortunately, Stan did the same for us. We left his group armed with the skills to succeed in virtually any position, for as Stan told us many times, "No matter what you end up doing, you've got to write."

We will explore Stan's research over the years, with special focus on his collaborative efforts with MSU faculty members as well as scientists at other institutions. We will feature some of his most interesting ideas and give an overview of all of his work.

(SPEC-11.2) Woodworking Science: Demystifying the Homemade Ebonizing Solution

Robert Q. Thompson¹, Robert Q. Thompson¹; ¹*Oberlin College*

One of the most commonly used sources of iron for ebonizing / darkening wood is the solution that results from the reaction of steel wool and vinegar. This iron solution has not-so-affectionately been called a "witches brew" and "liquid nightmare". The terms are apt, since many recipes exist, many different outcomes have been reported, and the whole process seems more magic than chemistry to woodworkers. We are the first to study carefully the reaction chemistry and to design a foolproof, reproducible recipe for preparing and using the resulting solution for ebonizing. We found (1) that the mass of steel wool to volume of vinegar ratio (1 g per 85 mL vinegar) is crucial to provide a final reaction solution mostly free of solids (remaining steel and precipitated product); (2) that inorganic phosphate acts as a catalyst for the reaction; (3) that the overall reaction is $\text{Fe(s)} + 2\text{CH}_3\text{COOH} \rightarrow \text{Fe}(\text{CH}_3\text{COO})_2 + \text{H}_2$; and (4) that the red-colored, diamond-shaped crystals produced contain both Fe(II) and Fe(III). Stan Crouch, whom we honor, was an analytical jack-of-all-trades: educator, electronic circuit builder, computer programmer, atomic

spectroscopist, expert on kinetics and flow methods, enzymologist, etc. In memory of his wide-ranging expertise, this presentation is a purposeful mix of analytical, bio-, inorganic, organic, and physical chemistry.

(SPEC-11.3) Novel, Autonomous, Microliter-scale, Integrated Sampling and Wet-Chemical-Analysis Platform for At-site Environmental, Industrial-Process, and Agricultural Monitoring

Charles J. Patton¹, Charles J. Patton¹, Curt Goodknight², Frank Goodknight; ¹*Segmented Solutions, LLC*, ²*Goodknight Consulting*

At-site monitoring of environmental and domestic-supply waters, industrial-process streams, and agricultural sites is increasing due to rising costs of vehicles and vehicle maintenance, fuel, and wages of skilled workers who collect samples, process them, and make them ready for transport to a laboratory. At-site deployment of commercially available sensors that measure pH, conductivity, turbidity, and dissolved oxygen is commonplace, but is far less so for analytes that lack sensitive and selective sensors—nitrogen- and phosphorus-containing plant nutrients, for example. This is because the cost and operational complexity of deployable instrumentation needed to sample, selectively derivatize, and detect resulting chromophores or fluorophores in complex matrices can be prohibitive. A game changing at-site sampling and wet chemical analysis platform is described below.

Novel features of the analytical module include a pump with 0.0625 μL per quarter step resolution that propels a water immiscible carrier stream bidirectionally through a length of $\approx 2\text{-mm}$ i.d. Teflon tubing serving as the analytical channel. The carrier stream is water-clear, has about twice the density of water, and wets Teflon resulting in near-zero carryover transport of entrained reagent-dosed samples (*assay boluses*). The analytical channel terminates in a 125-mL bottle that contains both the recirculated system fluid and aqueous analytical waste that accumulates immiscibly above it. Importantly, the sample inlet system—a 2-stage filter arrangement—is isolated from the analytical module by a 3-way rotary valve so that relatively large-volume sample rinse-in and rinse-out cycles do not contribute appreciably to the analytical module's waste stream—1,000 assays generate only about 75 mL of waste. Samples, reagents, calibrants, and a diluent connect to ports along the analytical channel through a series of normally closed rotary valves. Approximately 60- μL assay boluses are formed by programmed sequences of pump and valve commands. Resulting chromophores or fluorophores are measured radially across the analytical channel. Besides multipoint calibration and on-demand dilution, QC checks like duplicate analyses and sample spikes are also possible. This easy to operate and maintain, battery-powered platform with precise dispensing and a low-cost, high-performance photometer based on 20-bit LED driver and photodiode current digitizer circuits delivers laboratory-like precision and accuracy.

(SPEC-11.4) Anomalous Properties of Ionic Liquids. Using Fundamental Information to Advance Novel Applications

Gary J. Blanchard¹; ¹*Michigan State University*

Room Temperature Ionic Liquids (RTILs) hold much promise for use in areas ranging from energy storage and delivery to electronically-controlled optical devices and green synthesis. Despite the already wide use of these materials, a fundamental understanding of dynamics and properties such as the extent of dissociation in RTILs remains to be achieved. Our group has identified the existence of free charge density gradients in RTILs in contact with charged surfaces. These gradients can persist for ca. 100 nm in the RTIL and are manifested as gradients in the dielectric response of these materials. We provide an overview of these unique RTIL properties, the current state of our understanding, and some of the areas in which these properties can be of practical importance.

(SPEC-11.5) Applying the Concept of the Complete, Multi-step Analysis to Complex Health-related Problems: Lessons Learned in the Crouch Group

Dana Spence¹; ¹*Michigan State University*

I was trained in Stan Crouch's labs from 1992 to 1997. I was one of Stan's last couple of doctoral students and, from what I could gather, the Stan I worked for was a bit different than the Stan my predecessors knew. However, there were many similarities; specifically, Stan was passionate about research and teaching, attending conferences, and learning new things. He constantly encouraged me and the rest of our small group at the time, to pursue our own interests, as well as those of the group. However, it wasn't always pleasant. At the time, I didn't understand why Stan was adamant about learning "old school" topics such as mass balance of equations and chemical speciation; I was upset that Stan made me take a stats course in my 6th semester as a graduate student. He also made me take his microelectronics/automation course without asking if I wanted to take it. Now, 25 years post-graduation, I can look at my current projects in my group and honestly say we wouldn't have progressed to where we are without the skills I learned in those classes and during our group meetings with my doctoral advisor, Dr. Stan Crouch. I will provide a brief example of each of these skills being applied by my own group members as we work on our projects involving type 1 diabetes, multiple sclerosis and red blood cell transfusions.

22SPR04: Enhancing Chemical Processes with Plasmonics

Chair: Amanda Haes

(SPR-04.1) Assessing Plasmon Associated Electron Transfer

Zac D. Schultz¹; ¹*The Ohio State University*

The excitation of localized plasmon resonances has been shown to give rise to intense electric fields and energetic charge carriers on the surface of nanoparticles. Recent work suggests that some of the electrons excited by the plasmon resonance can be transferred to nearby molecules, altering the response observed from the molecules. In this presentation we will examine both ways to monitor the effects of these energetic charge carriers as well as the impact on the signals observed from the nearby molecules. Materials that take advantage of these effects suggest new opportunities in chemical analysis and other applications.

(SPR-04.2) Localized Surface Plasmon Resonance in Hydrogels

Francis P. Zamborini¹, Harikrishnan Nambiar; ¹*Louisville*

Here we describe the optical changes due to the localized surface plasmon resonance of metal nanoparticles (Au, Ag, or alloys) embedded into hydrogels. Metal nanoparticles of various size undergo different aggregation processes inside hydrogels similar to those in aqueous solution. This work explores the kinetics of the aggregation process and the optical changes that occur upon aggregation as compared to similar processes in water. Aggregation can be pH controlled, through electrostatic linking, heat, or chemical methods. The aggregation process is important to understand for potential sensing applications, controlling the optical properties of hydrogel materials, and for understanding the stability against aggregation for potential catalysis studies with the hydrogel serving as a support for the nanoparticle catalysts. The talk will focus on the fundamental aggregation process, optical properties, and potential applications.

(SPR-04.3) Spectroscopic Signatures of Plasmonic Hot Carrier Effects in the Steady State

Matt Sheldon¹, Matthew Sheldon¹; ¹*Texas A&M University*

Surface-enhanced Raman Spectroscopy (SERS) has been established as one of the most powerful modern analytical spectroscopic techniques. However, major challenges for further development of SERS for applications in analytical chemistry relate to: (1) difficulty in quantifying and reproducing stable Raman enhancement factors based on the SERS substrate design, (2) SERS substrates themselves contribute a spectrally broad “SERS background” signal of similar magnitude as absorbed analytes, in particular impeding quantitative analysis based on signal intensities, e.g. anti-Stokes thermometry, and (3) the strong optical field concentration provided by SERS substrates can promote photochemical and photothermal reactions that perturb the intrinsic chemical properties of analytes. At the same time, there has been significant growing interest to use the strong resonant absorption in plasmonic geometries to produce large transient populations of photo-excited non-equilibrium “hot” carriers that can then be employed in novel photochemical reactions.

This presentation will discuss how molecular SERS can be performed in parallel with quantitative analysis of the broad “SERS background”, and how this can enable new opportunities for resolving the challenges outlined above. Our laboratory has helped establish how this background signal is due to the electronic Raman (eR) response of the SERS substrate. Unlike more familiar Raman signals due to inelastic scattering of photons by specific molecular vibrational modes, the eR signal is characteristic of the entire energetic distribution of electrons in the SERS substrate.

In particular, CW electronic Raman spectroscopy during SERS can provide complimentary dynamical information compared with what can be learned from ultrafast transient absorption studies of plasmonic metals. Further, by analyzing the electronic Raman signal simultaneously with the conventional molecular Raman signal, our research shows how this expanded Raman methodology also provides unique chemical insights compared with other spectroscopic strategies, so that it may become a powerful resource especially for

understanding the dynamics of electron excitation, charge transfer, and sample thermalization during CW excitation of analytes on SERS substrates, even when there may be limited or ambiguous molecular indicators of the local photochemistry or chemical environment (e.g. no Raman-active species).

(SPR-04.4) Development of Highly Sensitive and Reproducible SERS Substrates

Jodie Fergusson¹, Stacey Laing², Sian Sloan-Dennison¹, Neil C. Shand³, Duncan Graham¹, Karen Faulds¹; ¹*The University of Strathclyde*, ²*University of Strathclyde*, ³*The Defence Science and Technology Laboratory (DSTL)*

Scaled-up, reproducible, "bright" SERS-active nanotags applied in a variety of applications and environments.

Gold nanoparticles are highly desirable in analytical applications due to their optical and physical properties; an example of this is the strong enhancement of Raman scattering of analytes they provide, due to their localised surface plasmon resonance (LSPR). This surface enhanced Raman scattering (SERS) property can be exploited to create nanotags with unique fingerprint Raman spectra that can be used to detect and identify molecules of interest.

Current research laboratory methods of synthesising gold nanoparticles utilise the Turkevich synthesis, resulting in small batches which is overall more time consuming, with large variation in properties such as size, charge and extinction. Scaling up the synthesis allows for highly reproducible nanoparticles, and this can be demonstrated via the characterisation of multiple 500 mL and 20 L batches of nanoparticles to identify variation. This reproducibility is an important consideration when designing assays which utilise gold nanoparticles, and minimal to no variation is a necessity to ensure accurate results which can be quantifiable.

Monodisperse gold nanoparticles have been produced from a 20 L synthesis and have been used as the core nanoparticles to create large batches of "bright" SERS active nanotags. This has been achieved by functionalising and aggregating the gold nanoparticles with a Raman reporter, to create hotspots, followed by a layer of silica to stabilise them. The resulting nanotags have a strong SERS intensity which is reproducible between batches, a common pitfall in SERS measurements. The ability to produce stable nanotags with constant SERS signals, on such a large scale, is desirable for a number of applications. These include altering the surface hydrophobicity of the nanotags via a modified Stöber process, allowing the tags to remain stable in a number of environments such as in biomatrices, and functionalising with antibodies for use in lateral flow immunoassay combined with SERS analysis, which could be implemented in the monitoring and prediction of chronic disease flare-ups. Additionally, their plasmon at ~ 785 nm allows for photothermal heating which suggests their suitability for use in tumour ablation, with monitoring of their position and activity within the body via SERS.

Friday October 7, 2022

22SCIFRI: SciFri Closing Plenary Session

Chair: Robert Lascola

22SCIFRI: SciFri Closing Plenary Session

(SCIFRI-01.1) Terrestrial Benefits of Space Exploration

Daniel Lockney¹; ¹NASA

NASA makes its technology portfolio available to promote commercialization and the public availability of Federally-owned inventions to benefit the national economy and the U.S. public.

The Space Agency has had a long history of finding new, innovative uses for its space and aeronautics technologies. This presentation will cover some of the historical highlights, recent examples, and provide guidance for accessing NASA's current portfolios.

(SCIFRI-01.2) The Spaceflight Environment and Human Health and Performance

Charles Doarn¹; ¹University of Cincinnati

From the dawn of human spaceflight, we have faced a wide variety of challenges that have been ameliorated over time but significant risks remain. The challenges include getting to space, providing the necessary tools to monitor crew health and performance, monitoring the environment (air, water, surface, radiation), crew training for all phases of flight, staying in space and eventually returning home. On board systems and telemedicine provide a level of comfort for immediacy juxtaposed to long duration / distance flight, which will be autonomous. Aerospace medicine physicians, life scientist and engineers are never on the same page when it comes human systems integration. They are trained differently and look at things with diverse constructs. The very first flights by the Soviets and the Americans were to prove it could be done; that humans could survive. In the 21st century, individuals, who are paying a lot of money are now traveling into space; some even staying at the International Space Station (ISS). Much has been learned about the physiological impact as well as toxicology and microbiology of the environment, and the psychological impact to the human system. This presentation will cover the many attributes of human space exploration, which will include health systems, environmental monitoring and the future exploration initiatives of Artemis, Gateway, Mars and the remaining years of the ISS. International participation and commercial programs will be touched as well.

(SCIFRI-01.3) From Ocean Worlds to the Big Blue: How Planetary Robotics is Helping Us Explore the Deep Sea Cost-Effectively

Pablo Sobron¹; ¹Impossible Sensing

