Biomaterial Interfaces
Room 101A - Session BI+AS-MoM

Biomolecules and Cells at Interfaces
Moderator: Joe Baio, Oregon State University

8:20am **BI+AS-MoM1 Probing the Selectivity of Antimicrobial Peptides to Cell Membranes by Sum Frequency Generation Spectroscopy**, ***Thaddeus Golbek,*** Oregon State University*; J. Franz,* Department of Molecular Spectroscopy, Max Planck Institute for Polymer Research, Mainz, Germany*; J.E. Fowler, K.F. Schilke,* Oregon State University*; T. Weidner,* Department of Molecular Spectroscopy, Max Planck Institute for Polymer Research, Mainz, Germany*; J.E. Baio,* Oregon State University

Cationic amphiphilic peptides have been engineered to target both Gram-positive and Gram-negative bacteria while avoiding lysis of other cell types. However, the exact mechanism of how these peptides target, bind, and disrupt bacterial cell membranes is not understood. One specific peptide that has been shown to selectively capture bacteria is WLBU2 (sequence RRWVRRVRRWVRRVVRVVRRWVRR). It has been suggested that WLBU2 activity stems from the fact that when interacting with bacterial cell membranes the peptide assumes an α-helical structure and inserts itself into the membrane. To test this hypothesis, we applied sum frequency generation (SFG) spectroscopy and surface tensiometry to probe the peptide-lipid-air interface and identify the structure and monitor the interaction of WLBU2 with two model lipid monolayers that mimic mammalian and bacterial cell membranes. Model mammalian cell membranes were built upon zwitterionic 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) lipids while bacterial cell membranes were constructed with negatively charged 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (DMPG) lipids. The rate at which the surface pressure reaches equilibrium is 4.3 times faster for WLBU2 interacting with the negatively charged DMPG lipid monolayer than with the zwitterionic DPPC lipid monolayer. This observed WLBU2 binding affinity preference to negatively charged membranes is likely due to electrostatic interactions between positively charged amino acids within the peptide and negatively charged lipids. SFG studies at the peptide-lipid-air interface demonstrate that binding of WLBU2 induces increased lipid monolayer order. A larger increase in acyl chain order from 2.2 to 3.4 determined by the ratio of the CD_3 symmetric (2075 cm^{-1}) and CD_2 symmetric (2100 cm^{-1}) peak amplitudes suggest that WLBU2 is found at the surface of the zwitterionic phospholipid monolayer and not inserted. The amide I region SFG spectrum of WLBU2 interacting with the zwitterionic lipid monolayer shows two peaks near 1642 cm^{-1} and 1678 cm^{-1} indicative of an inactive β-sheet structure. A peak near 1651 cm^{-1} for WLBU2 interacting with negatively charged lipids is assigned to an active α-helix structure. Altogether, we demonstrate that WLBU2 shows a higher binding affinity to bacterial cell membranes and is in an active α-helix structure, alternatively in the presence of mammalian cell membranes in an inert β-sheet structure.

8:40am **BI+AS-MoM2 Bacterial Adhesion to Immobilized Liquid Layers under Dynamic Conditions**, ***Caitlin Howell,*** University of Maine*; Y. Kovalenko, I. Sotiri,* Harvard University*; J. Overton,* University of Maine*; J. Aizenberg,* Harvard University

Immobilized liquid (IL) layers are an emerging technology shown to prevent bacterial biofouling of surfaces. In this work, we show how in one class of IL-coated materials, infused polymers, bacterial adhesion can be strongly dependent on growth conditions. Samples grown with *Escherichia coli* under more relevant dynamic conditions showed significantly increased colony-forming unit counts compared to the same system grown under static conditions. Direct visualization of the surfaces suggested that this was due to a disturbance of the IL layer when exposed to shaking conditions, which allowed more bacteria to remain on the surface after an initial rinse. However, no incorporation of the bacteria into the oil layer was detected. To further investigate the extent of this adhesion, we used sequential removal cycles to gauge the relative adhesion strength of the remaining surface-bound *E. coli*. Through this method, we found that despite no initial difference in adherent CFUs compared to control samples with no IL layers, IL samples did reduce overall adhesion of the bacteria even after incubation under dynamic conditions. Further tests on a flagella-deficient strain of *E. coli* revealed that while flagella play a significant role in adhesion to IL layers, they are not the sole adhesion mechanism for this species. Finally, tests on two other clinically-relevant species of bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, using similar methods revealed clear species-dependent differences in adhesion after growth under dynamic conditions. These results shed new light on the interaction of bacteria with IL layers, and demonstrate the importance of both relevant growth conditions and thorough analysis to obtaining clear results in these systems.

9:00am **BI+AS-MoM3 Nitric Oxide Materials—An Approach to Creating More Hemocompatible Medical Device Coatings**, ***Hitesh Handa,*** University of Georgia **INVITED**

Blood/material interaction is critical to the success of implantable medical devices, ranging from simple catheters, stents and grafts, to complex extracorporeal artificial organs which are used in thousands of patients every day. There are two major limiting factors to clinical application of blood contacting materials: 1) platelet activation leading to thrombosis, and 2) infection. Despite a thorough understanding of the mechanisms of blood–surface interactions, and decades of bioengineering research effort, the ideal non-thrombogenic prosthetic surface remains an unsolved problem. One approach to improving the hemocompatibility of blood-contacting devices is to develop materials that release nitric oxide (NO), a known potent inhibitor of platelet adhesion/activation and also an antimicrobial agent. Healthy endothelial cells exhibit a NO flux of 0.5-4x10^{-10} mol cm^{-2} min^{-1}, and materials that mimic this NO release are expected to have similar anti-thrombotic properties. I will discuss the potential of incorporating NO donor molecules such as diazeniumdiolates or S-nitrosothiols (RSNOs) into various polymers, and their hemocompatibility and antibacterial properties in short-term (4 h) and long-term (7 d) animal models.

9:40am **BI+AS-MoM5 Why do Bacteria Stick to Some Surfaces and Not Others? Characterisation of the Behaviour of Motile Bacteria at and Above the Surface of Materials**, *A.L. Hook, A. Carabelli, N.A. Russell, P. Williams,* ***Morgan Alexander,*** The University of Nottingham, UK

High throughput screening has been used to discover a novel class of polymers with resistance to bacterial attachment and subsequent biofilm formation.[1,2] Physicochemical descriptions of the surfaces have to date been found insufficient to predict the wide range of bacterial attachment across these diverse polymer libraries, and cannot offer an explanation of the controlling phenomena. Whilst perhaps disappointing for the physical sciences, the life sciences are replete with information on how bacteria respond to their local environment, with chemotaxis being one of the most readily observed processes. Unsurprisingly, microorganisms cannot be approximated to inert spheres and rods as they possess surface responsive appendages such as flagella, which enable them to swim, pili that confer twitching motility and fimbriae that mediate surface attachment. These in turn are coupled to sophisticated signal transduction mechanisms that facilitate integration of multiple local environmental parameters at both single cell and population levels. Many of these sensory systems are postulated to contribute to surface sensing. As an example of the complexity of these processes, the opportunistic pathogen *Pseudomonasaeruginosa* has over 60 two-component sensor kinase response regulator systems involved in environmental adaptation.

We believe that bacterial decision-making is key to determining whether a surface is colonised or not. I will present the early results from our optical microscopy investigations of how individual bacterial cells respond to surfaces. We have developed a novel microscope that collects temporal 3D information on cell position using both holography and remote scanning microscopy. [3] Simultaneously surface tracking can be achieved using DIC, TIRF and TIR microscopy. This allows us to track not only the motion of single cells at the surface, but also their approach to and behaviour after contact with the surface.

We will combine these findings with our existing understanding of the surface chemistry-attachment relationships achieved for certain subsets of materials and attachment regimes,[4,5] with chemical analysis of the in situ surface to build a complete description of this complex biointerface and the response of bacteria to it. This information is crucial in determining how bacteria behave with respect to defined surfaces and has important implications for the prevention of device centred infections.

1. Hook et al. Nature Biotechnology 2012

2. Hook et al. Advanced Materials 2013

3. Botcherby et al. Circulation Research 2013

4. Epa et al. Advanced Functional Materials 2014

5. Sanni et al. Advanced Healthcare Materials 2015

10:00am **BI+AS-MoM6 Probing Adhesion of Marine Biofilm Formers by Microfluidics**, *K. Nolte,* Ruhr-University Bochum, Germany*; M. Alles, M.P. Arpa-Sancet, C. Christophis,* University of Heidelberg, Germany*;* ***Axel Rosenhahn,*** Ruhr-University Bochum, Germany

When new Materials are developed to control and influence Biofilm growth, the ability of biofilm formers to firmly adhere to the coatings is one key property. Several techniques have been developed in the past to probe attachment strength of cells [1]. Especially microfluidic test systems [2] offer several advantages, such as small sample area, small amounts of target species, and high throughput. We developed microfluidic assays that allow to test bacterial and diatom adhesion on coatings [3,4]. Cells are driven through a microchannel at a precisely controlled flow rate and at a constant concentration and both, accumulation and detachment can be monitored by video microscopy. Using self-assembled monolayers as model surfaces we were able to show that the adhesion strength correlates with the accumulation dynamics if an appropriate shear stress is applied. Based on this finding, a parallelized microfluidic system has been developed that allows simultaneous, comparative testing of materials. Due to the modular assembly of the setup, not only model surfaces and thin organic films, but also practical coatings can be analyzed.

[1] L. Marcotte, M. Tabrizian, ITBM-RBM 2008, 29, 77

[2] D.P. Bakker, A. van der Plaats, G.J. Verkerke, H.J. Busscher, H.C. van der Mei, Appl. Envir. Microbiol. 2003, 69(10), 6280

[3] M. Arpa-Sancet, C. Christophis, A. Rosenhahn, Biointerphases 2012, 7, 2

[4] M. Alles, A. Rosenhahn, Biofouling. 2015, 31, 469–480.

10:40am **BI+AS-MoM8 Protein Control of Materials Nucleation Probed by Sum Frequency Generation***,* ***Tobias Weidner,*** Max Planck Institute for Polymer Research, Mainz, Germany

Proteins can act as Nature's engineers at interfaces and manipulate both hard and soft tissue – they shape biominerals, manipulate cell membranes and nucleate materials. Despite the apparent importance for engineers working in the fields of surface engineering, drug delivery, or diagnostics, the molecular mechanisms dictating interfacial protein action have remained largely elusive. Our goal is to probe the structure and structural dynamics of such active proteins – in action at the surface.

Mineral proteins have the ability to control and steer the growth of hard tissue by binding specific mineral facets and precipitating silica and phosphates. They control the intricate mineral morphologies found in diatom cell walls, mollusk nacre, but also human teeth and bone. Inspired by diatom silification we used amphiphilic peptides consisting of leucine and lysine (LK peptides) to investigate biomineralization at surfaces. These peptides can adopt helical or beta sheet structures at the air-water interface. Upon addition of a silica precursor we obtained freestanding peptide-silica hybrid sheets with thicknesses of ~4 nm. We have followed the biomineral composition and interactions between peptides and silica at different early stages of biomineralization using a combination of surface spectroscopies and microscopies. Our experimental findings were complemented with molecular dynamics simulations. Our data shows that the peptide surface folding dictates the nanometer scale morphology of the prepared silica film.[1]

A particularly fascinating example of protein driven nucleation and phase transitions are ice-nucleating proteins. These proteins are used by specific bacteria to attack plants and cause frost damage by growing ice crystals at temperatures that would otherwise not allow ice formation. A recent survey by the NASA found large amounts of biological ice nucleators in the troposphere where they may affect global precipitation patterns. We have followed the interaction of freeze proteins with surrounding water molecules – how specialized protein sites lock water molecules in place and manipulate the flow of energy within the surrounding layers of water.[2]

1 H. Lutz, V. Jaeger, R. Berger, M. Bonn, J. Pfaendtner, T. Weidner

Biomimetic growth of ultrathin silica sheets using artificial amphiphilic peptides

Advanced Materials Interfaces, 1500282 (2015).

2 R. Pandey, K. Usui, R. A. Livingstone, S. A. Fischer, J. Pfaendtner, E. H. G. Backus, Y. Nagata, J. Fröhlich-Nowoisky, L. Schmüser, S. Mauri, J. F. Scheel, D. A. Knopf, U. Pöschl, M. Bonn, T. Weidner

Ice-nucleating bacteria control the order and dynamics of interfacial water

Science Advances, 2 (2016).

11:20am **BI+AS-MoM10 Regulation of Cell Surface Access and Mechanics at the Interface***,* ***Jennifer Curtis,*** *P. Chang, W. Wei, L.T. McLane,* Georgia Institute of Technology*; J. Scrimgeour,* Clarkson University **INVITED**

A polymer brush-like structure decorates the cell surface of many cell types ranging from fibroblasts to mesenchymal stem cells to cancer cells. This sugar-rich pericellular matrix (PCM) plays physical and chemical roles in biological processes ranging from brain plasticity, to adhesion dependent processes like cell migration, to the onset of cancer. Here I will report on biophysical and mechanical assays that characterize the structure of the pericellular matrix and its impact on the transport of nanoparticles and molecules to the cell surface. Further, I will present compelling quantitative evidence that hyaluronan polymer expression at the cell-substrate interface tunes cell adhesion strength, working in concert with focal adhesions.

Applied Surface Science
Room 101B - Session AS+BI-MoA

Practical Surface Analysis I: Advancing Biological Surface Analysis/Imaging Beyond 'Show and Tell'

Moderators: Ian S. Gilmore, National Physical Laboratory, UK, Jordan Lerach, The Pennsylvania State University

1:40pm **AS+BI-MoA1 A Multi-technique Approach for Studying the Effect of Protein G B1 Orientation on Antibody Binding***, Elisa Harrison, G. Interlandi, D.G. Castner,* University of Washington

The orientation of adsorbed proteins on surfaces plays a vital role in the function and performance of biomaterials. Development of diagnostic tools such as sandwich ELISAs have focused on controlling the orientation of each protein layer. A full understanding of adsorbed proteins on surfaces, especially at the molecular level, is therefore essential. Our research address es the challenges for characterizing protein orientation by developing new method s to study multilayer protein systems.

The aims of this study were to control and characterize the orientation of protein G B1, an IgG antibody-binding domain of protein G, on well-defined surfaces and measure the effect of its orientation on antibody binding using a variety of surface-sensitive tools and simulations. We hypothesize that binding selectivity would increase for well-ordered protein films due to high er availability of binding domains.

The surface sensitivity of time-of-flight secondary ion mass spectrometry (ToF-SIMS) enables us to distinguish between different proteins and their orientation by monitoring the changes in intensity of amino acid mass fragments. We have developed ToF-SIMS methods for analy zing the orientation of five different cysteine mutants of protein G B1 covalently attached to a maleimide surface. T his technique was further extended by studying multilayer protein systems, specifically the binding of IgG antibodies to the protein G B1 films.

To study the effect of protein orientation on antibody binding, we utilized self-assembled monolayers (SAMs) to form protein G B1 films with both random and well-defined orientations. Using complementary techniques, such as X-ray photoelectron spectroscopy and quartz crystal microbalance with dissipation monitoring (QCMD), the ratio of bound IgG antibodies to protein G B1 increased from 0.06, when chemisorbed onto bare gold, to 0.2, when covalent ly attach ed to the surface. Further analysis revealed structure/orientation rearrangement of protein G B1 upon adsorption onto bare gold, which is likely responsible for decreased antibody binding.

Additionally, we developed and applied Monte Carlo (MC) simulations to predict protein orientation on a surface. The MC simulations showed that the outermost b-sheet of protein G B1 interacts most frequently with a hydrophobic surface. The predicted orientations were verified using molecular dynamics simulations, QCMD, and sum frequency generation.

The model systems explored in this study are a first step in developing methodology using state-of-the-art tools that can be applied to more complex systems and expand our knowledge and control of biomolecules on surfaces.

2:00pm **AS+BI-MoA2 ME-SIMS Revisited: Attempting to Unlock the Potential using Advancements in Sample Preparation and SIMS Technology***, Nina Ogrinc Potocnik,* Maastricht University, The Netherlands*; C.R. Anderton, L. Pasa-Tolic,* Pacific Northwest National Laboratory*; R.M.A. Heeren,* Maastricht University, The Netherlands

This year marks the 20th anniversary of Wu and Odom first describing the application of a solid organic matrix to improve the ionization efficiency of molecular species in secondary ion mass spectrometry (SIMS) measurements. This so-called matrix enhanced-SIMS (or ME-SIMS) method overcame one of the disadvantages of SIMS analysis, providing the capability of imaging large molecules with high spatial resolution. With increased ionization efficiency and minimized fragmentation caused by the primary ion beam, the method is ideal for detection of intact bimolecular species, where detection of proteins greater than 10,000 Da is feasible. However, the combination of instrumentation limitations of resolving isobaric compounds and lateral diffusion caused by matrix application has pushed this technique into near irreverence. Here, we reevaluate ME-SIMS with new technologies such as parallel MS/MS capabilities on the PHI nano-TOF TRIFT V, and the custom-build FTICR-SIMS capable of unmatched mass resolving power and mass accuracy. We also explore new matrix application techniques to revisit the potential of ME-SIMS and apply it to a number of different biological settings.

Specifically, we reexamined peptide standard profiling with the addition of tandem MS on the nano-TOF TRIFT V . The ability to isolate precursor ions with a 1 Da mass window, followed by a high-energy collision-induced dissociation (CID), enables a very precise fragmentation of molecules. We observe peptide fragmentation through the amino terminus, a_m, providing us with a specific fragmentation pattern for identification of peptide species and opening doors to *de novo* peptide sequencing. Further on, we applied it for characterizing tryptically digested peptides investigating the applicability to bottom-up proteomics. We then imaged model plant and mammalian tissue sections that were subjected to a variety of different matrices *via* supplication using a home-built sublimation chamber. Matrix sublimation produces small, homogenous crystal sizes, without the need for solvents that delocalize molecular species. Consecutive sections were analyzed by FTICR-SIMS, to accurately identify molecular species of interest, and by the nano-TOF TRIFT V for high lateral resolution images and confident identification of said species with tandem MS.

2:20pm **AS+BI-MoA3 Improvements in SIMS Methods and Instrumentation in Effort to Make Measurements Biologists Can Use***, Christopher R. Anderton,* Pacific Northwest National Laboratory **INVITED**

The ability of mass spectrometry imaging (MSI) to visualize chemical distributions within samples has made it an increasing popular method in many biological fields, including medicine, pathology, and microbial ecology. Secondary ion mass spectrometry (SIMS) is a surface sensitive MSI technique that offers extensive versatility in its ionization and analysis modes, requires relatively minimal preparation, and can achieve the highest lateral resolution of any MSI method. Early bio-applications of SIMS routinely focused on pursuing the molecular information attainable by softer ionization methods (e.g., matrix assisted laser desorption/ionization), but with the added benefit of achieving subcellular lateral resolution. Even though primary ion beams used in SIMS measurements afford smaller probing areas than other ionization methods, their excessive energy typically causes extensive fragmentation of most biorevelent molecules. This renders identification of parent molecules from the detected secondary ions a nontrivial endeavor. Nevertheless, recent improvements in SIMS instrumentation, methods, and data analysis approaches have unlocked biochemical information that was previously unattainable. Here, I will discuss our efforts in improvements in sample preparation methods and the employment of unique mass spectrometer technology for analyzing biological material. Stable isotope probes were used to decode lipid distributions within model and cellular membranes, to reveal the intercellular delivery of drug-loaded polymeric nanoparticles, and to elucidate metabolic processes of phototrophic communities. The use of Fourier transform-based mass spectrometers, which have unparalleled mass accuracy and mass resolving power, and tandem mass spectrometry methods have allowed us to unravel the extreme spectral complexity of biological SIMS measurements, while increasing the confidence in our measurements. Lastly, we have revisited previously reported sample preparation routes that were never fully adapted by the SIMS community, in part because they were shackled by the limited ability of more commonly employed mass analyzers.

3:00pm **AS+BI-MoA5 Towards Bacterial Differentiation with Quantitative SIMS***, Christopher Szakal, S. Da Silva,* National Institute of Standards and Technology (NIST)*; N. Olson,* National Institute of Standards and Technology(NIST)

Large geometry secondary ion mass spectrometry (LG-SIMS) has been used extensively for particle analyses and geochemical analyses, owing to its ability to maintain adequate mass resolution while operating at high secondary ion transmission. Efforts will be presented that extend the knowledge acquired in these application areas to single bacterial cell analyses of elemental species. To be useful, LG-SIMS results need to be quantitative for the amounts of a given element per cell and/or in ratios of different elements within each cell. Approaching this level of detail requires the establishment of the natural variability of such data from cell-to-cell, the reproducibility of the measurement technique, and whether the data is relevant to pertinent questions about the cellular population. Progress will be shown towards achieving these aims for single bacterial cells within different known growth conditions, including analytical figures of merit for LG-SIMS elemental ratios. Prospective application areas will be presented, along with potential pitfalls of such an approach.

3:20pm **AS+BI-MoA6 New Insights into the Microenvironment of Cancerous Tissue by Combined Mass Spectrometry, Microscopy and Multivariate Analysis**, ***Tina Angerer,*** University of Gothenburg, Sweden*; Y. Magnusson, G. Landberg,* Sahlgrenska Cancer Center, Sweden*; J.S. Fletcher,* University of Gothenburg, Sweden

Introduction

Mass spectrometric imaging is of growing interest for the medical field, both in applied and basic research[1]. Particularly, imaging secondary ion mass spectrometry (SIMS) is becoming of increasing value to clinicians and has been used on a number of tissues samples to successfully identify and localize different chemical components to various areas of the tissue and answer disease related questions[2]. Fatty Acid Synthase (FAS) has been shown to be increased in many cancer types and is of growing interest as therapeutic target[3]. The changed lipid composition due to increased FAS activity is an ideal ToF-SIMS study target.

Methods

With the J105- *3D Chemical Imager* (Ionoptika Ltd), fitted with a 40 kV gas cluster ion gun[4], we are now able to overcome some previous limitations of ToF-SIMS analysis and image large intact molecular species at high spatial and high mass resolution simultaneously. To capitalize on these improved capabilities we performed imaging SIMS on fresh frozen hydrated and freeze dried, ductal mammary breast cancer sections, followed by H&E staining of the analysed sections.

Results

SIMS enables us to distinguish between different areas of the diseased tissue. Multivariate analysis facilitates localizing and grouping the up to 10,000 different signals generated from the tissue to produce comprehensive chemical profiles assigned to different areas in the tissue revealing underlying structures. We have identified a number of molecules which can be, due to high spatial resolution, clearly assigned to the cancerous regions, characterized by conventional histological staining, in different breast cancer sections. Additionally, studying the distribution of specific single ions reveals reoccurring patterns of changes and gradients within the cancerous areas which cannot be observed in the conventionally stained image. Therefore ToF-SIMS can provide deeper insights into tumor metabolism and progression. Our results agree with findings from experiments using different methods, which confirm these molecules to be cancer markers while more importantly elucidating new information form the tissue with cellular resolution.

Conclusions

Imaging ToF-SIMS is a valuable tool for cancer research and can provide new insights into chemical changes within tumors. Further application of ToF-SIMS imaging will be used to study different modes of disease progression and treatment response.

[1] J. L. Norris et al., *Proteom Clin Appl* **2013**, 7, 733-738.

[2] A. Brunelle et al., *Curr Pharm Design* **2007**, 13, 3335-3343.

[3] P. M. Alli, et al., *Oncogene* **2004**, *24*, 39-46.

[4] T. B. Angerer et al., *Int J Mass Spectrom* **2015**, 377, 591-598.

4:00pm **AS+BI-MoA8 Super-resolution Mass Spectrometry Imaging of Biological Materials with the New 3D nanoSIMS**, ***Ian S. Gilmore,*** *M.K. Passarelli,* National Physical Laboratory, UK*; A. Pirkl, R. Moellers, E. Niehuis,* ION-TOF GmbH, Germany*; A.A. Makarov,* Thermo Fisher Scientific*; H.F. Arlinghaus,* ION-TOF GmbH, Germany*; R. Havelund, P.D. Rakowska, A.M. Race, A.G. Shard,* National Physical Laboratory, UK*; A. West,* GlaxoSmithKline*; S. Horning,* Thermo Fisher Scientific*; P. Marshall,* GlaxoSmithKline*; M.R. Alexander,* The University of Nottingham, UK*; C.T. Dollery,* GlaxoSmithKline

SIMS has become an important technique for the surface analysis of biological materials. However, critical challenges have hampered the uptake into the life-science industry and biomedical discovery. To succeed in this important sector, it has to progress beyond "Show and Tell". Biological samples have complex chemistry and an extraordinarily large dynamic range of concentration. The present state-of-the-art struggles to identify unknowns owing to insufficient mass resolving power and mass accuracy of time-of-flight analysers. The situation is further complicated by sample form and vacuum compatibility.

To address this issue, we have developed a powerful new hybrid SIMS instrument combining an Orbitrap™-based Thermo Scientific™ Q Exactive™ HF instrument and a dedicated ToF-SIMS 5. The instrument is equipped with high-resolution ion beams including a new micron resolution argon cluster ion beam for biomolecular imaging and 3D analysis of organics and an ultra-high resolution Bi cluster focussed ion beam with < 80 nm resolution. The ToF analyser allows high-speed imaging needed for 3D analysis and the High Field Orbitrap analyser allows high mass resolution, mass accuracy and MS/MS for chemical identification. The instrument is designed for life-sciences applications including sub-cellular 3D imaging of metabolites, imaging of bacteria and biofilms and imaging of medical devices with complex topographies that confound traditional instrument designs.

We show data demonstrating the unique advantages of this novel instrument. Imaging with large argon clusters provides rich biomolecular spectra including intact lipids and metabolites. Existing state-of-the-art instruments are limited to a mass resolving power of around 6,000 which is insufficient to allow unique identification. We show images of mouse brain with a sub-cellular spatial resolution of less than 2 microns simultaneously with a mass resolving power of over 100,000 for intact lipids. We fully separate the (3'-sulfo)Gal-Cer(d18:1/24:1(2-OH)) and (3'-sulfo)Gal-Cer(d18:1/25:0) sulfatides, which reveals a difference in spatial distribution. In the low mass region, mass resolving powers of >400,000 are achieved allowing clear separation of the low abundance metabolite dopamine from other peaks. We show the ability to image the drug amiodarone with sub-cellular resolution and show that the mass spectra are not affected by sample topography. The instrument is also equipped with state-of-the-art cryogenic sample preparation specifically designed for high-resolution biological imaging.

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986

4:20pm **AS+BI-MoA9 High-resolution, Sub-cellular Imaging of Pharmaceutical Localization by Correlative SIMS and TEM**, ***Paulina Rakowska,*** National Physical Laboratory, UK*; H. Jiang,* University of Western Australia*; I.S. Gilmore,* National Physical Laboratory, UK

To accurately predict the pharmacological effect of potential drug candidates, there is a strong need in the pharmaceutical industry to image the disposition of drugs at the sub-cellular level and even within specific organelles. This is needed to answer long-standing questions about whether drug concentrations are sufficiently high in the right places to have a therapeutic effect, or if the medicine is lodging within cellular components and causing toxicity. If anomalies were spotted earlier, it might help to explain toxicities or lack of efficacy of a medicine and reduce costly late-stage failures.

Mass spectrometry imaging techniques are well-suited to measure drug distribution in biological samples and have the advantage of label-free analysis. The CAMECA nanoSIMS (secondary ion mass spectrometry) can provide elemental images with high lateral resolution of 50 nm. These high-resolution ion images can be correlated to electron microscopy images. This combination of techniques provides very precise and detailed information of cell morphology, subcellular processes and localization of different molecules within the cells. However, these high-performance instruments require high vacuum and complex sample preparations. Therefore, the sample handling needs careful consideration. Biological samples can suffer from ultrastructural reorganization or the loss or translocation of molecules, which can occur with dehydration under high-vacuum conditions. Chemical fixation of the samples followed by embedding in resin are common in the studies of cell biology by TEM but the solvents used for sample dehydration have a severe effect, translocating or even removing the drug from the cell all together. This has been a fundamental barrier for the use of the technique for intracellular drug localization measurement.

We present a correlative nanoSIMS and TEM imaging of a highly lipophilic drug – amiodarone within lung macrophages dosed at therapeutic concentrations. The protocol used for the fixation and resin-embedding of the cells prevented the drug from being removed from the organelles during solvent treatment. We are able to show, with unprecedented detail, the drug accumulating in lysosome organelles.

4:40pm **AS+BI-MoA10 Sub-Micron Imaging and Identification of Molecular Chemistry by TOF-SIMS Parallel Imaging MS/MS**, ***Gregory Fisher****,* Physical Electronics*; N. Ogrinc Potocnik, A.L. Bruinen,* Maastricht University, The Netherlands*; J.S. Hammond, S.R. Bryan,* Physical Electronics*; R.M.A. Heeren,* Maastricht University*; S. Iida, T. Miyayama,* ULVAC-PHI **INVITED**

A recently introduced TOF-TOF imaging mass spectrometer allows conventional TOF-SIMS (MS^1) analysis and product ion (MS^2) analysis to be

* **ASSD Peter Sherwood Award**

achieved simultaneously and in parallel. Secondary ions for MS^1 and MS^2 analysis are produced from the same area of the surface by a pulsed and digitally raster-scanned primary ion nanoprobe. The sensitivity of the parallel imaging MS/MS spectrometer is high so that the analytical ion dose may be minimized; therefore, precious and one-of-a-kind samples may be probed without significant damage or degradation. Fragmentation of the molecular precursor ions, defined by a 1 Da precursor selection window, is accomplished by collision-induced dissociation (CID) at 1.5 keV in an activation cell of Ar gas at high pressure. Lateral resolutions produced in both MS^1 and MS^2 images are demonstrated to be in the range of 100 nm < $\Delta l_{80/20}$ < 1 µm. This tandem MS imaging capability has been brought to bear for straightforward identification as well as multifaceted studies involving biological, material, and polymer specimens. We will summarize here some of our ongoing biological research, revealing molecular identification at sub-micron practical lateral resolution.

One study concerns song bird ontogeny in male zebra finch (*T. guttata*). Several sulfatides, phospholipids, sterols and fatty acids have been identified as playing a role in song learning. We have employed parallel imaging MS/MS to unravel the roles of specific molecules because the shortcomings of TOF-SIMS imaging alone does not permit conclusive molecular identification and imaging. We have evidence suggesting that distinct sulfatides are active primarily within the song nuclei while cholesterol and specific fatty acids are active in signaling between the song nuclei.

In other work, we have probed the role of lipids and metabolites in disease states of zebrafish (*D. rerio*) that have been infected with *M. marinum*, a form of tuberculosis. The bacteria initiate a granulomatous inflammation, and first signs of the disease are observed in the spleen. We have observed so far that α-tocopherol is elevated in infected tissue as well as in the granuloma, but is not present in the necrotic cells. Cholesterol is elevated primarily in the granuloma. The role of phospholipids appears to differ, specific molecules being either elevated or depressed in the infected tissue. We have preliminary evidence of a metabolic source for bacterial growth. For example, we observe a phosphocholine, PC(16:0/16:0), to be elevated in the granuloma. However, in the necrotic cells surrounding the granuloma we observe elevated signals of a fatty acid, FA(16:0).

Plasma Processing for Biomedical Applications Focus Topic Room 101A - Session PB+BI+PS-MoA

Plasma Processing of Biomaterials

Moderators: Denis Dowling, University College Dublin, Deborah O'Connell, University of York, UK

2:00pm **PB+BI+PS-MoA2 Atmospheric Plasma Deposition of Antimicrobial Nano-Coatings on Biomedical Textiles***, A. Nikiforov, I. Kuchakova, T. Coenye, C. Leys,* Ghent University, Belgium*; N. Hojnik, M. Modic,* ***Uroš Cvelbar****,* Jozef Stefan Institute, Slovenia

In this work, the antimicrobial non-woven fabrics were prepared with the use of atmospheric pressure plasma deposition.Atmospheric pressure DC jet operating in N_2 at current density of 6 mA/cm^2 and voltage of 15 kV is used as a source of non-thermal plasma for engineering of the antibacterial nano-composites on surface of polymeric polyethylene terephthalate (PET) meshes. Nano-particles of Ag, Cu and ZnO are tested as antimicrobial agents through incorporation in to the structure of the plasma deposited composite film. The deposition process is carried out in three steps process. The fabric is first pretreated by depositing a first layer (250 nm - 500 nm) of organosilicon thin film using an atmospheric pressure plasma system, then nano-particles are incorporated by a dipping-dry, and finally the nano-particles are covered by a second organosilicon layer of 10-50 nm thickness. Top layer in the composite coating of "sandwich-like structure" with variable thickness is used for precise control of metal ions release and so to tune antimicrobial efficiency of the material. The deposition process and surface chemistry of the coatings are studied by emission spectroscopy, and surface analysis techniques: XPS, AFM and SEM. The antimicrobial activity of the treated fabrics is also tested against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It is revealed that thickness of top (barrier) layer plays a key role in release of metal ions and negligible small antibacterial activity is observed if barrier thickness exceeds 50 nm. Tests with *S. aureus* show that the highest 98% bacterial reduction is achieved with Cu NPs whereas Ag NPs are much less effective and can provide only 79% reduction. In contrast, the fabric antibacterial efficiency against of *Pseudomonas aeruginosa* is very low for both Cu and ZnO nanoparticles in spite of the load and only Ag NPs are proved to be effective (2 orders reduction) against of *P. Aeruginosa*. The results clearly indicate that plasma of atmospheric pressure can be used as effective tool for immobilization of nano-particles in composite coatings. Control of antibacterial activity can be achieved through variation of deposition parameters and a type of incorporate nanoparticles. The approach might present a new route to preparation of effective antimicrobial materials against of certain class of bacteria.

This work is partially supported by the M.Era-Net project "PlasmaTex".

2:20pm **PB+BI+PS-MoA3 Plasma Polymers for Biomedical Applications***,* ***Farzaneh Arefi-Khonsari****,* l'université Pierre et Marie Curie, France*; A. Baitukha, J. Pulpytel, A. Valinataj Omran,* Sorbonne Universités, UPMC, France **INVITED**

In this talk, different nonequilibrium atmospheric pressure plasmas used for biomedical applications such as planar DBD, single and double barrier DBD plasma jets, and transported discharges in tubes will be discussed. Indeed in the case of the latter, deposition and surface treatment, by means of a He cold transported discharge in tubes as long as 200 cm and tube inner diameters ranging from 1 to 20 mm, can present a great potential for surface modification of polymers used as biomaterials. We have, as well as several research groups, succeeded to retain the precursor moieties to obtain PEG like polymers which present interesting antifouling properties by using planar DBD and jets. However for particular plasma applications such as making a Drug Delivery System (DDS) based on several polymer or copolymer layers, encapsulating the drug, it is more reasonable to use a low pressure plasma which can give rise to dense crosslinked barrier films. The latter are less flexible and develop microcracks due to swelling and curvature of the host biocompatible and biodegradable substrate. In order to obtain good cohesive coatings with excellent barrier and mechanical properties, it is very important to deposit layers presenting a vertical chemical gradient, where stress is gradually distributed over the rigid and flexible zones of the DDS, which is more easily deposited in low pressure plasmas. Our recent results in copolymerizing amphiphilic precursors for the use of cell adhesive or nonadhesive surfaces will be presented. Such copolymers can be also used as biodegradable multi-layer copolymers for drug delivery applications. Human ovarian carcinoma cell lines (NIH:OVCAR–3) were used for *in vitro* measurements of cell interactions with the surface of fabricated DDS. Proposed model of DDS on collagen films prevents migration, adhesion and growth of cancer cells on its surface, and by tuning the thickness of the dense barrier films, encapsulating the drug, it is possible to control the drug release kinetics and to improve the therapeutic effect. *In vivo* experiments were carried out by injecting OVCAR3 cells in mice lymph nodes to develop a tumor, followed by implantation of the DDS membranes to evaluate the feasibility of the proposed model.

3:00pm **PB+BI+PS-MoA5 Plasma Coating Using Biologics: Degradation or Polymerisation?***,* ***Liam O'Neill****, J. O'Donoghue,* TheraDep, Ireland **INVITED**

The interaction of plasma with biomolecules is generally viewed as being a simple degradation reaction in which the plasma denatures any biologic material it encounters. Using a combination of heat, UV, free radicals, electrons and ions from the plasma, it is possible to cut, oxidise, burn and even ablate biological materials and this has established plasma sterilisation as a trusted technique in science, medicine and engineering.

However, recent research in our labs has shown that it is possible to minimise these effects and to instead use the plasma to cross-link biologic materials with retention of the biological properties of the precursor materials. Using low levels of applied plasma power, it is possible to produce low energy helium and argon plasma discharges. When biomolecules are nebulised into such a low temperature plasma, the materials are activated without losing their chemical structure. This activation can then effectively cross-link or coagulate the biomolecule without significant degradation. In addition, the plasma can activate substrates and effectively bind the biomolecules to the substrate as a thin nano-scale coating.

The result is a one-step process capable of modifying the surface of medical devices, research and diagnostic lab ware, implants and even living tissue. Tailored biological surfaces can be grown in situ over large areas using established equipment systems. The mechanisms used to control such reactions and to move the plasma from degradation to cross-linking modes are now being established and will be discussed. Examples of protein and polysaccharide coatings produced to date will also be presented.

4:00pm **PB+BI+PS-MoA8 Low and Atmospheric Pressure Plasma Polymerization for Immunosensing and Tissue Engineering**, ***Lenka Zajickova***, *A. Manakhov, E. Makhneva, J. Medalova, D. Necas,* Masaryk University, Czech Republic*; L. Strbkova,* Brno University of Technology, Czech Republic*; A. Obrusnik, M. Landova,* Masaryk University, Czech Republic **INVITED**

Plasma polymerization provides a large playground for the preparation of surfaces suitable for immobilization of biomolecules and colonization by cells because chemical, structural and functional properties of plasma polymerized thin films can be tuned accordingly. The key decision for the particular application is the selection of functional chemical group that the final plasma polymer should contain. This contribution is going to discuss deposition of plasma polymers containing amine and carboxyl groups, functional groups that are typically used in biochemical applications and that are proposed to influence positively the attachment and proliferation of cells at surfaces. Amine-rich films were deposited in the low pressure pulsed radio frequency discharge using vapors of cyclopropylamine mixed with argon. The films contained primary and secondary amines and a small amount of oxygen. The structure of the films, reflected in their stability in water, could be tuned by the plasma conditions. The relationship between the amount of amine groups and the water stability was not straitforward because the films with similar amount of primary amine groups but different cross-linking could be prepared. The plasma polymers containing anhydride groups that hydrolyzed fastly at air into carboxyl groups were deposited in kHz-frequency dielectric barrier discharge at atmospheric pressure from the mixture of maleic anhydride and acetylene. The variation of the flow rate ratio was used to optimize the stability of films together with the amount of functional groups. Amine and carboxyl plasma polymers proved to be useful for the preparation of immunosensors based either on the principle of quartz crystal microbalance or surface plasmon resonance because in both these methods it is necessary to prepare a stable and reactive film on the gold surface. The amine films were also tested for the cultivation of human dermal fibroblasts and mouse myoblasts. It was identified that the water stability of the films is very important for succesfull experiments

4:40pm **PB+BI+PS-MoA10 Low-Temperature Plasma Processing of Polymeric Materials for Biomedical Applications**, ***Michelle Mann***, *M.R. Maynard, E.R. Fisher,* Colorado State University

Polymeric biomaterials are widely used in medical applications such as wound healing, drug release, and blood dialysis. For example, Tygon® and similar thermoplastics are chosen for these applications because of excellent mechanical strength and flexibility but often suffer from bacterial attachment and proliferation that ultimately leads to infection and fouling of the biomedical device. Biocidal agents can be incorporated into the polymer to actively eradicate bacteria, but it is difficult to ensure that biocidal action is localized at the material-biological interface. As a result, changing the surface properties of the polymer ensures a second mechanism by which to discourage bacterial attachment and growth. Plasmas are frequently used to alter the surfaces of biomaterials, most often by surface modification or deposition of a film to discourage bacterial attachment, while retaining the bulk properties critical to device performance. Specifically, H_2O (v) plasma treatment can enhance the compatibility of biomaterials by increasing hydrophilicity and altering surface chemistry; here, we demonstrate the use of this treatment method specifically for antibacterial materials. First, we have used H_2O (v) plasmas to tune the release of an antibacterial agent (NO) from drug-releasing polymers. Composition of treated drug-releasing polymers measured via X-ray photoelectron spectroscopy demonstrates a 100% increase in oxygen content and an associated increase in wettability, as observed via water contact angle goniometry. Compared to the untreated polymer, H_2O (v) plasma treated polymers had a delayed, but equally dramatic 8-log reduction in growth of both gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*. Second, in a related study, we utilized plasma-enhanced chemical vapor deposition to deposit a film of 1,8-cineole, an antibacterial constituent of tea tree oil. Bacterial attachment and biofilm formation assays reveal significantly reduced growth of both bacterial strains on plasma polymerized cineole films. H_2O (v) plasma treatment of these materials will also be discussed. Furthermore, optical emission spectroscopy allows correlation of gas phase excited state species in our plasmas under various plasma conditions to the resulting 1,8-cineole film surface properties, thereby allowing for fine-tuning of film surface properties for deposition onto biomedically-relevant polymer structures such as 3D polycaprolactone scaffolds. Collectively, our studies of plasma processing of antibacterial materials demonstrate this technique is a valuable tool in the production of next generation biomedical devices.

5:00pm **PB+BI+PS-MoA11 Plasma-based Functionalization of Polystyrene Surfaces of Cell Culture Plates**, ***Kazuma Nishiyama***, *T. Ito, S. Sugimoto, K. Gotoh, M. Isobe,* Osaka University, Japan*; M. Okamoto,* Osaka University Hospital, Japan*; A. Myoui,* Osaka University Hospital, Japanl*; H. Yoshikawa, S. Hamaguchi,* Osaka University, Japan

Polystyrene is one of the most widely used cell-culture plate materials. Amino and/or carboxyl coated cell culture plates are commercially available and such surface functionalizations are known to contribute effectively to the control of growth and differentiation of various stem cells. Plasma-enhanced chemical vapor deposition (PECVD) or plasma ion implantation may be used to functionalize polystyrene surfaces of cell culture dishes. The goal of this research is to understand how such surface functionalizations are affected by plasma conditions. In this study, we have used molecular dynamics (MD) simulation to understand how incident ions and free radicals affect the formation of amines and carboxyl groups. The simulation is based on interatomic reactive potential functions developed in-house based on quantum mechanical calculations. Results of MD simulations under the conditions similar to PE-CVD by ammonia (NH3), cyclopropylamine (CPA), or N2/CH3OH plasmas or ion implantation by NH3, N2/H2, or N2/CH3OH plasmas suggest that, with energetic ion bombardment, functional groups such as primary amines are less likely to form and nitridation of the surface tends to occur. Some simulation results have been compared with experimental data obtained from parallel-plate discharges with an inverter power supply at a relatively high gas pressure of 250 - 2,500 Pa and found to be in good quantitative agreement.

Plasma Processing for Biomedical Applications Focus Topic
Room 101A - Session PB+BI+PS-TuM

Plasma Processing of Biological/Biomimetic Surfaces

Moderators: Uroš Cvelbar, Jozef Stefan Institute, Slovenia, Satoshi Hamaguchi, Osaka University, Japan

8:00am **PB+BI+PS-TuM1 Investigation of Discharge Propagation on Cell and Plasmid Suspension in Plasma Gene Transfection**, ***Yugo Kido***, Pearl Kogyo Co., Ltd., Japan; *H. Motomura, Y. Ikeda,* Ehime University, Japan; *S. Satoh,* Y's Corp., Japan; *M. Jinno,* Ehime University, Japan

The authors have been developing a plasma gene transfection technique and averaged transfection efficiency up to 20% and cell survivability up to 90% are achieved for more than 20 kinds of cells. A typical procedure of this method is as follows. Target cells are cultured on a 96 well plate and gene suspension is added. The plate is placed between a high voltage electrode made of copper capillary with the diameter of 70 µm and a copper plate grounded electrode. By exposing the suspension to a microplasma generated at the tip of the capillary electrode, the cells are transfected by the genes. In this method, both chemically reactive species (chemical factors) and discharge current (electrical factors) are indispensable to the transfection process and their synergistic effect has been experimentally verified. Moreover, the transfection occurs on the whole area of each well although only the central area is exposed to the microplasma. In this study, to clarify how the discharge current contributes the transfection process with the synergistic effect with the chemical factor, discharge propagation phenomenon on the cell and plasmid suspension is investigated.

As a target, COS-7 cells are cultured on a 35 mm dish and 24 µg of plasmid pCX-EGFP suspended in 120 µL of TE/PBS buffer is added. The applied voltage between the electrodes is 20 kHz sinusoidal waveform and the amplitude is set at 10 kV peak to peak. The gap length between the capillary electrode and the suspension is set at 1 to 5 mm. The discharge propagation is observed with an ICCD camera equipped with a UV lens.

When only TE/PBS buffer solution exists in the dish, the discharges reach the buffer solution surface and then they propagate radially up to 15-20 mm of diameter. On the other hand, when the cells are cultured on the dish, remarkable radial propagation of the discharge is not observed and the discharge irradiation area is limited within the diameter of about 1 mm, which is narrow compared with the area in which the transfection occurs. Therefore, as a contribution of the electrical factors, not only the direct effect of the discharge current, charge on the plasmids, conduction current in the solution etc. should be analyzed. As a first step of the chemical factor investigation, similar observation is performed by the ICCD camera with an interference filter to observe the emission of OH radicals. The results of the discharge propagation paths study by means of equivalent circuit simulation and comparative analysis between the discharge propagation and the transfection will be shown at the symposium.

8:20am **PB+BI+PS-TuM2 Spectroscopic Study of Permeability of Stratum Corneum by Plasma Treatment for Transdermal Drug Delivery**, ***Jaroslav Kristof***, *N. Tran, M. Blajan, K. Shimizu,* Shizuoka University, Japan

Application of drugs by needles presents risk of infections and causes pain. On the other side, oral application of drugs can be toxic for human body because drug has to be transported through alimentary tract and higher amount of active agent is required. Transdermal delivery could be ideal painless and effective way but barrier function of skin has to be reduced for improving permeability of drugs. Research of last years proves that plasma can interact with skin and cause decreasing barrier function of skin [1-3].

We used plasma jet and microplasma discharge for investigation of barrier function of stratum corneum – horny layer of of Yucatan micropig skin. Helium or argon was used as working gas. These rare gases were later enriched by liquids like water or ethanol through the bubbling system to achieve higher amount of active particles like OH.

Physical changes of the pig skin were observed by microscope. As the human body is not non-conductive, we can expect different results when conductivity of layer under skin is changed. We compared effect of plasma on conductive and non-conductive material. Placement of skin on conductive material caused burned spots on skin by plasma jet [3]. While it was isolated, no damage was observed with plasma jet irradiation. In case of treatment of skin by microplasma, physical damage was hardly observed.

Changes in stratum corneum layer were observed by Attenuated Total Reflectance – Fourier Transform InfraRed (ATR-FTIR) spectroscopy. ATR-FTIR spectrum offer information about water, lipid bilayer and proteins in stratum corneum. Permeability of skin for drugs correlates with shift of asymmetric stretch of CH_2 band to higher wavenumbers. This information describes behavior of lipid bilayer. Information about reaction of proteins on plasma treatment content Amide I and Amide II bands. Reaction of stratum corneum layer of pig skin depended on used discharge type and gas. Effectivity of plasma sources and used gases or gas mixtures for transdermal drug delivery was analysed.

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8:40am **PB+BI+PS-TuM3 Cell Attachment to Microwave Plasma-oxidized Titanium and Titanium Alloy Substrates**, ***Denis Dowling***, University College Dublin, Ireland; *M. Naciri,* University Mohamed V of Rabat, Morocco; *M. Al-Rubeai, A. Breen,* University College Dublin, Ireland **INVITED**

Titanium and its alloys have been widely investigated for use in orthopedic and dental implant devices, particularly for osteointegration and bio-compatibility. This paper evaluates the influence of titanium surface oxidation using a microwave plasma treatment technique on cell attachment. Commercially pure titanium (CpTi) and titanium alloy (Ti6Al4V) discs were treated in an oxygen atmosphere for 5 min-utes at 850 °C using a microwave (2.45 GHz) plasma system, operating at 2 kPa. After the 5-minute treatment, the thickness of the oxidized layer was 2.3 µm on the CpTi discs and 4.7 µm on the Ti6Al4V discs, with growth rates of 0.5 and 1 $\mu m.min^{-1}$ respectively. Reduced plasma oxidation rates were observed on a high surface area beaded surface (Porocoat). In contrast to the plasma treatments, the use of air furnace oxidation only achieved an oxide layer thickness for the CpTi of 1 µm, when treated at the same temperature. Optical profilometry measurements were performed to determine the surface roughness; XRD, EDX, and SEM examinations were also car-ried out to determine the properties of the oxide layers and their morphologies. Cell attachment to the treated discs was also assessed after exposure times of 25 and 100 minutes. A 40% increase in MG63 osteoblast cell attachment on the Ti6Al4V discs was observed, when compared with that on the CpTi discs. Alkaline phosphatase (ALP) specific activity of MG63 cells grown on control and plasma oxidised surfaces were compared after 21 days. A statistically significant difference between Ti6Al4V and CpTi oxidised surfaces ($P<0.05$), when compared to that obtained for the control surface that had not been plasma treated. The acicular morphology of the oxidised Ti6Al4V surface was found to have the most significant influence on enhancing cell attachment, combined with higher oxide layer roughness and thickness.

9:20am **PB+BI+PS-TuM5 The Role of Electrical and Chemical Factors in the Molecular/Gene Transfection by Micro-Plasma Irradiation**, ***Masafumi Jinno***, *Y. Ikeda, H. Motomura,* Ehime University, Japan; *Y. Kido,* Pearl Kogyo Co. Ltd., Japan; *S. Satoh,* Y's Crop., Japan **INVITED**

The plasma gene transfection is expected as a safe and useful method of gene transfection. However, this method had a problem of a difficulty in keeping both high transfection efficiency and less cell damage simultaneously. The authors have evaluated four different plasma sources, such as arc discharge, plasma jet, DBD (dielectric barrier discharge) and microplasma, in terms of the transfection efficiency and the cell viability. High transfection efficiency is achieved by the styles of arc discharge and microplasma in which the electric current flows via the cells. Our experimental results suggests that an electric current may play an important role in plasma gene transfection, and that total volume of the gas flow must be small or zero and the area in which the cells are directly irradiated by plasma must be small in order to achieve higher cell viability. Among the various types of plasmas, which the authors have tried, the microplasma satisfies these conditions and brings both the high transfection efficiency and the high cell viability simultaneously.

We evaluated the contribution weight of three groups of the effects and processes inducing gene transfection, i.e. electrical, chemical and biochemical ones through three experiments. The laser produced plasma (LPP) was employed to estimate the contribution of the chemical factors. The liposomes were fabricated and employed to evaluate the effects of plasma irradiation on membrane under the condition without biochemical

reaction. The clathrin-dependent endocytosis, one of the biochemical processes was suppressed. It also turned out the clathrin-dependent endocytosis is the process of the transfection against the 60% in all the transfected cells. The endocytosis and electrical poration are dominant in plasma gene transfection, and neither permeation through ion channels nor chemical poration is dominant processes.

By scavenging the H_2O_2 generated by plasma irradiation using catalase, the transfection efficiency decreased to 40% of that of without catalase. On the other hand, when the H_2O_2 solution is dropped in the cell suspension without plasma irradiation, the transfection is not observed. These results suggest that the synergistic effect of H_2O_2 with electrical factors or with other reactive species generated by plasma irradiation is important. Consequently it becomes clear that chemical factors, radicals such as H_2O_2 and reactive oxygen/nitrogen species, do not work by itself alone, and that the electrical factors (electrical current, charge and field) are essential to plasma gene transfection.

11:00am **PB+BI+PS-TuM10 Control of Plant Growth by RONS Produced Using Nonthermal Atmospheric Air Plasma**, ***Kazunori Koga,*** Kyushu University, Japan; *T. Sarinont,* Kyushu University; *M. Shiratani,* Kyushu University, Japan

Nonthermal atmospheric plasmas have been widely used for biomedical applications because of their non-equilibrium feature and synergy effects [1-3]. The non-equilibrium feature allows us to introduce reactive oxygen and nitrogen species (RONS) to biomaterials with a significantly wide dose range compared with the conventional irradiation methods such as X-ray and γ-ray [4]. For an agricultural application, we succeeded in reducing harvest period and enhancing crop yield by plasma irradiation to plant seeds [5]. We found irradiation of RONS with an appropriate dose to seeds brings about growth enhancement in all growth stages of plants. To understand the growth enhancement mechanism, here we have studied dependence of irradiation dose of RONS produced by plasma to seeds on growth of *Arabidopsis thaliana L.* Experiments were carried out using a scalable DBD device [2, 3, 5]. The device consisted of 20 electrodes of a stainless rod of 1 mm in outer diameter and 60 mm in length covered with a ceramic tube of 2 mm in outer diameter. The discharge voltage and current were 9.2 kV and 0.2 A. 20 seeds of Arabidopsis thaliana L. were set 3 mm below the electrodes. The RONS dose was controlled by the irradiation time. After plasma irradiation, they were grown on soil tab in incubators. To evaluate plant growth, the stem length was measured as a function of cultivation days. The stem length was normalized by the stem length of the plants without plasma irradiation. To evaluate statics of the measured values, we used a two-tailed ANOVA statistically significance different at $\alpha = 0.05$ ($p < 0.05$). The normalized stem length increases to 1.3 for 3 min irradiation, then decreases to zero for 10 min irradiation. The results indicate the plant growth is activated by plasma irradiation less than 3 min and inactivated by plasma irradiation of 5-10 min. Above 10 min irradiation, no seeds were germinated. We have succeeded in growth control of plants from death to activation with irradiation dose of RONS produced by plasma. The mechanism will be discussed in the presentation.

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11:20am **PB+BI+PS-TuM11 Generation of Reactive Species in Medium Irradiated Laser-Induced-Plasmas**, ***Yukihiro Kurokawa,*** *N. Kurake, K. Takeda, K. Ishikawa, H. Hashizume, H. Tanaka, H. Kondo, M. Sekine, M. Hori,* Nagoya University, Japan

The non-equilibrium atmospheric pressure plasma (NEAPP) was irradiated to the cell culture medium as liquid. The antitumor effect, showing the selective killing effect for cancer cells without killing normal human cells, was reported [1,2]. This effect are considered to be caused by large amounts of reactive nitrogen and oxygen species (RONS) generated by the plasma. However, chemical reactions during transport of plasma in ambient to the liquid surface is complicated; therefore we have applied the laser-induced plasma.

Previously, we reported that the high ratio of NO_2^-/ H_2O_2, even in low H_2O_2 contained in the plasma activated medium [3]. However, relations of reactive species concentrations with antitumor effects have not been fully elucidated. Here, we focus on the concentrations of reactive species generated in culture media by the laser-induced plasma.

A Nd:YAG laser and harmonic generators (Quanta Ray Pro 230, Spectra Physics) provided the pulsed-laser light with a wavelength of 266 nm, a frequency of 30 Hz, a power at sample surface of 25 mW. The light was focused on the gas-liquid interface of ultrapure water or Dulbecco's Modified eagle Medium (DMEM; cat. no. 5796; Sigma) by using plano-convex lens, made of synthetic quartz. 2 mL of the liquid was typically irradiated for 5 min. This is called as LPAM. Just after irradiation, H_2O_2 and NO_2^- concentrations were measured by using absorption that was measured by ultraviolet-visible near infrared spectrometer (V-650, JASCO). Moreover, HeLa cells were incubated in the LPAM and cell survival was measured after 24 h incubation. For analysis of killing mechanism, activated caspase3/7 as apoptosis marker (CellEvent Caspase-3/7) was measured after fluorescent staining by a fluorescent microscope.

The LPAM generated effectively H_2O_2 causing by photo-dissociation of water, hydroxyl radicals (・OH) works a precursor of H_2O_2 with the reaction of ・OH + ・OH →H_2O_2. Survival of HeLa cells in the LPAM was dependent on dilution of the LPAM and standard DMEM. We prepared the diluted LPAM for a half of killing of HeLa cells. After the cultivation for 24 h in the diluted LPAM, the caspase-3/7 activity of dead cells as apoptosis death was observed clearly. Notably, the cell-death was almost inhibited by catalase.

We will discuss on the generation mechanism of active species and the mechanism of antitumor effect of the LPAM with comparison of the PAM.

This work was partly supported by MEXT KAKENHI on Innovative Areas Grant no. 24108002.

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11:40am **PB+BI+PS-TuM12 Electric Fields in kHz-driven Plasma Jets**, *ET. Slikboer, Y.N. Nguyen,* Eindhoven University of Technology, The Netherlands; *O.Y.N. Guaitella,* Ecole Polytechnique, Palaiseau, France; *G. Sretenović,* University of Belgrade; *A. Obrusnik,* Masaryk University, Brno; ***Ana Sobota,*** Eindhoven University of Technology, The Netherlands **INVITED**

Non-thermal atmospheric pressure plasma jets have been developed for use on thermosensitive targets at atmospheric pressure, for example polymers or for biomedical applications. Diagnostics on these plasma sources is challenging because of their transient nature, often associated jitter and very small volume. Electric fields, fundamental property essential for the understanding of the discharge, are not well known. In this talk two methods of electric field measurements will be shown applied to a He kHz-driven jet, one based on spectroscopy and one on polarimetry and the obtained results will be discussed.

Biomaterial Interfaces
Room 101A - Session BI+AS+SA-TuA
Biophysics and Characterization of Biological and Biomaterial Surfaces

Moderators: Eva Chi, University of New Mexico, Axel Rosenhahn, Ruhr-University Bochum, Germany

2:20pm **BI+AS+SA-TuA1 Resolving Non-specific and Specific Adhesive Interactions of Catechols at Solid/Liquid Interfaces at the Single Molecular Scale**, *T. Utzig,* Max-Planck Institut für Eisenforschung GmbH, Germany*; P. Stock,* Max Planck Institut fur Eisenforschung GmbH, Germany*;* ***Markus Valtiner****,* Technische Universität Freiberg, Germany

The adhesive system of mussels evolved into a powerful and adaptive system with affinity to a wide range of surfaces. It is widely known that thereby 3,4-dihydroxyphenylalanine (Dopa) plays a central role. However underlying binding energies remain unknown at the single molecular scale. Here, we use single molecule force spectroscopy to estimate binding energies and binding mechanism of single catechols with a large range of opposing chemical functionalities. Our data demonstrates significant interactions of Dopa with all functionalities, yet most interactions fall within the medium-strong range of 10-20 k_BT. Specifically, Dopa-molecules interact with surfaces exposing different functionalities via different types of interactions ranging from bidentate H-bonding plus metal coordination (titania), monodentate H-bonding (SAMs exposing H-donor or H-acceptor headgroups), the hydrophobic interaction (alkyl SAM) or interactions involving the p-electron system of Dopa's catechol ring (gold). Only bi-dentate binding to TiO_2 surfaces exhibits a higher binding energy of 29 k_BT. Our data also demonstrates at the single molecule level that oxidized Dopa and amines exhibit interaction energies in the range of covalent bonds, confirming the important role of Dopa for cross-linking in the bulk mussel adhesive. We anticipate that our approach and data will further advance the understanding of biologic and technologic adhesives.

2:40pm **BI+AS+SA-TuA2 Protein-Nanoparticles Interactions: Surface Chemistry, Protein Corona and Secondary Structural Changes**, *I. Ojea, R. Capomaccio, L. Calzolai, D. Gilliland, P. Colpo,* ***Giacomo Ceccone****,* EC-JRC-IHCP, Italy*; G. Siligardi, R. Hussein,* Diamond Light Source, Oxfordshire, UK

The characterisation of protein corona formed around nanoparticles is a very important and challenging issue in the investigation of nanomaterials behaviour in biological environment and has been studied by many authors [1, 2, 3,4].

On the other hand, it is recognized that detailed physico-chemical characterization of nanomaterials is becoming increasingly important both from the technological and from health and safety point of view. Moreover, an incomplete characterisation may inhibit or delay the scientific and technological impact of nanoscience and nanotechnology [5]. In this respect, surface chemical analysis methods, such as X-ray Photoelectron Spectroscopy (XPS) and Time of Flight Secondary Ion Mass Spectrometry, can provide an important contribution to more fully characterizing nanomaterials [6].

In this work, we have investigated the interaction of human serum albumin (HSA) with gold nanoparticles (AuNPs) functionalized with thiols. In particular, 15 nm AuNPs functionalized with PEG thiols have been studied before and after interaction with HSA.

The different steps of sample preparation have been characterised by DLS, CPS and TEM, whilst the surface chemistry has been mainly assessed by XPS. Finally, the interaction between nanoparticles and HSA has been studied by Synchrotron Radiation Circular Dichroism (SRCD) to gather information on the protein structure [7]. In particular, XPS and ToF-SIMS data revealed the presence of HSA on pegylated nanoparticles, whilst the use of SRCD in combination with separation techniques allowed the determination of the structure and morphology of HSA-AuNPs complexes [8]. Moreover, SRCD experiments indicate that AuNPs increase the UV and thermal stability of HSA.

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3:00pm **BI+AS+SA-TuA3 Measuring the Impact of the Surface of Protein Stability using Single Molecule Experiments with the AFM**, ***Phil Williams****, S. Allen, A. Oyefeso, G. Milson, E. Fornari,* University of Nottingham, UK **INVITED**

Seven out of the top eight top-selling medicines of 2014 were biological in origin (so-called biopharmaceuticals or biologics). Successful formulation of such biopharmaceuticals has created new challenges to the pharmaceutical industry since the physical and chemical properties of the biological molecule (protein, peptide, RNA, DNA) differ from those of small 'classical drug' molecules. Whilst single molecule force spectroscopy has given new insight to many ligand/receptor interactions, the requirement to chemically functionalize the surfaces of both the substrate and the tip render the technique of little interest to the pharmaceutical industry since such functionalization, by definition, changes the chemistry of the ligand and receptor. Furthermore, this experimental methodology precludes effective screening of agents binding to a target receptor.

I will highlight our development of a fragment screening methodology using the AFM for single molecule force measurements without chemical modification of the ligands. I will introduce the method validating its approach using the streptavidin/biotin system that is often used as a model. I will then demonstrate the potential of the methodology to find fragments that interact with thrombin, a target for cardiovascular disease therapy.

In developing the above approach, it became apparent that actually neither the tip nor the substrate needs to be functionalized. I will conclude by discussing a promising method to screen for excipients that may stabilize protein structure in formulation and storage, where no chemical functionalization is necessary. The technique permits the measurement of the stability of proteins to be measured through their susceptibility to denaturants, such as urea and guanidinium chloride, and the effect of excipients on the measured stability to be assessed. For some proteins, the stability measured through traditional bulk methods, such as fluorescence, match those measured using the AFM, whereas for others there appears to be a significant difference. I propose, therefore, that this AFM method offers an interesting way to study protein denaturation at an interface.

4:20pm **BI+AS+SA-TuA7 In Vitro Characterization of Interfaces for the Development of Antibacterial and Biocompatible Surfaces**, ***Katharina Maniura****,* Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland **INVITED**

Cell culture and bacterial studies of novel materials and new functional surfaces often show very poor correlation with clinical outcomes. This fact not only poses a major challenge for basic and industrial researchers, it is also associated with high costs.

Generally, the majority of biomaterials are tested using *in vitro* cell monocultures, however, this approach neglects possible synergistic interactions between different cell types and paracrine signalling mediating the tissue-specific response to a material.

Immediately upon implantation, medical implants get exposed to the patient's blood and this initiates the first phase of wound healing and subsequent cell recruitment and response deciding about material integration or non-integration.

We have established that blood pre-incubation of implant surfaces mimics a more physiological situation, providing a more predictive *in vitro* model for the evaluation of novel implant surfaces.

Similarly, many promising antimicrobial materials failed to make the translation from bench to bedside, partially due to insufficient *in vitro* biofilm models used for predicting the long-term *in vivo* antimicrobial and anti-biofilm activity. For the evaluation of novel surfaces the actual forseen implantation location and its biological environment need be considered to design a more predictive bacterial study with conditions mimicking the *in vivo* situation.

5:00pm **BI+AS+SA-TuA9 Vibrational Sum-Frequency Scattering Spectroscopy for Characterization of Biomaterial Interfaces in Biological Environments**, ***Patrik Johansson****, C. McDonald, Y.-C. Wang, P. Koelsch, D.G. Castner,* University of Washington

Most biomaterials have a 3-dimensional structure, of which the interfacial properties play an essential role in their interactions with biomolecules in the surrounding environment. The dynamics of protein adsorption onto biomaterials, and the induced conformational changes or selective

orientations following such interactions, are phenomena that to a large extent govern the biocompatibility of such materials. However, direct measurement of these interactions in biological environments are challenging as most techniques often (1) lack interfacial specificity, (2) require model samples with inherent limitations, or (3) lack specificity for the chemistry, orientation, and conformation of the probed species. In this work, we demonstrate how vibrational sum-frequency scattering (SFS) can be used to provide all this information, without the use of labels, from biomolecules specifically at the surface of biomaterials in biological environments.

We first show that SFS can yield chemical information via vibrational spectra selectively from molecules used to functionalize the surface of nanoparticles. Spectral changes upon addition of proteins to the samples do not only confirm adsorption onto the nanoparticles, but also provide information about the secondary conformation for the adsorbed proteins. It is likely that continuous development of SFS will make it an essential tool for evaluating the biocompatibility and other properties of nanoparticles for use in biomedical applications.

We have also applied SFS on protein fibers, for which a detailed understanding of the structure, function, interactions, conformation, and dynamics is critical for refining strategies in tissue engineering, as well as for the development of treatments for progressive diseases involving protein fibers, such as Alzheimer's disease (AD). In our studies, we have found that collagen fibers assembled *in vitro* exhibit a very large SFS cross-section, and that the spectral signatures are dependent on the scattering angle, implying that this parameter can be adjusted to selectively study specific features of the fibers. Data analysis routines, including maximum entropy method calculations, reveal the relative phase of various chemical groups in the fibers, which can be utilized for determining their relative orientations.

Finally, we have demonstrated that amyloid fibers and spherulites, which are structures found in the brain tissue of patients with AD, exhibit strong nonlinear optical properties. We believe that SFS can reveal new details about the development and interactions of these structures, which can provide clues about AD pathology and help finding new biomarkers for the disease.

5:20pm **BI+AS+SA-TuA10 Imaging ToF-SIMS of Human Breast Cancer Tissues: Connecting Chemical Images to Biology**, ***Blake Bluestein,*** University of Washington; *F. Morrish, D. Hockenbery,* Fred Hutchinson Cancer Research Center; *L.J. Gamble,* University of Washington

Breast cancer, the most common cancer among women, is known to vary in responsiveness to chemotherapy. Therefore, the role of changes in tumor metabolism affecting the response to chemotherapy is under scrutiny. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) provides a powerful approach to attain spatially-resolved molecular data from cancerous tissues. We use imaging ToF-SIMS and principal components analysis (PCA) to study human biopsy tissue samples to clarify links between fatty acid composition within and around tumors and the potential drug resistance of these tumors. An important component of this project is ToF-SIMS analysis of pre and post neoadjuvant frozen patient specimens. Since treatment occurs with the tumor in place, analysis of biopsies taken pre- and post-treatment allows characterization of molecular changes in tumors as a response to treatment. Two sets of pre and post chemotherapeutic treated tissue have been studied. Additionally, 11 triple negative (TN) pre-treatment tissues have been studied using PCA to determine if molecular differences within tumor tissues can be correlated with patient response to treatment.

Data were acquired with an IONTOF TOF.SIMS V using a Bi_3^+ analysis beam. Multiple 1mm^2 areas per tissue section were analyzed by stitching together 25 200µm^2 raster area scans. Data was acquired in both positive and negative polarities. Scores images generated by imaging PCA correlated with cellular and stromal areas were then used as masks to select regions of interest (ROI) that were reconstructed with ToF-SIMS software. Reconstructed spectral data of cellular and stromal areas was subsequently analyzed using PCA to ascertain molecular differences between tumor tissues.

Utilizing ROIs to select specific regions within analysis areas followed by spectral PCA for two different sets of pre and post treatment tumor biopsies showed a near distinctive chemical separation between pre and post. Chemical differences observed between the pre and post treatment tissue biopsies were related to changes in fatty acids, monoacylglycerols, diacylglycerols and cholesterol. Pretreatment samples showed higher loadings for vitamin E and C18:1 while post treatment samples had higher loadings for sphingomyelin and saturated fatty acids (stearic acid and palmitic acid). Spectral PCA of cellular and stromal region data from the 11 TN tissues separates patients that respond to chemotherapy and those that do not. Patients that respond to chemotherapy show higher loadings of sphingomyelin and saturated fatty acids, while nonresponding patients correlate with loadings of cholesterol, C18:1 and C18:2.

5:40pm **BI+AS+SA-TuA11 Some of These Images are Just Like the Others: Finding Similar Images in Imaging Mass Spectrometry Data Sets**, ***Daniel Graham,*** *L.J. Gamble,* University of Washington

Mass spectrometry imaging (MSI) has been applied to many areas of research due to the rich chemical information it can provide. However, MSI also brings a set of challenges due to the enormous size of the data sets. Most modern imaging mass spectrometers produce data that consists of a full mass spectrum at every pixel of each image. This data set can be analyzed either as a series of spectra from a given area of the image, or as a series of images from a given set of peak masses. When looking at a series images, it is of interest to find all masses that have the same spatial distribution since this could provide information about the chemical differences seen throughout a sample, and identify fragments that originate from the same molecules or that co-localize within the analyzed area. In this presentation we demonstrate a simple, useful tool we have developed to process mass spectrometry images and identify which peaks show similar spatial patterns. For this we have created the 'Correlated Image Finder' as part of our NBtoolbox for multivariate analysis of mass spectrometry imaging data. This tool uses one of two methods to find similar images. The first method calculates the correlation coefficient between the pixels of each image and sorts the images according to a user chosen correlation cutoff. The second method uses a simple image subtraction method to find images that match within a user chosen cutoff. For either method, the images are first down binned to reduce image noise and then thresholded and scaled in order to compare all peak images on an equal scale.

The Correlated Image Finder has been tested on a wide variety of images. Examples will be shown from ToF-SIMS and MALDI imaging data. It was seen that the Correlated Image Finder is able to find images showing similar spatial distributions. The Correlated Image Finder can be used on any set of image data and examples will be shown from both 2D and 3D image data sets from tissues, cells and polymers. The results from the Correlated Image Finder can help simplify MSI data interpretation and can also help understand trends seen using other analysis methods such as principal component analysis.

Novel Trends in Synchrotron and FEL-Based Analysis Focus Topic

Room 103C - Session SA+AS+BI+MI-TuA

Synchrotron and XFEL Advances for Biological Systems (2:20-3:40 pm)/Synchrotron Radiation at the Frontiers of Device Technology (4:20-6:20 pm)

Moderators: David Shuh, Lawrence Berkeley National Laboratory, Olivier Renault, CEA-University Grenoble Alps, France

2:20pm **SA+AS+BI+MI-TuA1 Crystal Growth Mechanisms of Biominerals Revealed by Polarization-dependent Imaging Contrast (PIC) Mapping**, ***Pupa Gilbert,*** University of Wisconsin - Madison **INVITED**

X-ray linear dichroism was first shown in natural biominerals by Metzler et al. [1]. Based on this effect, we developed Polarization-dependent Imaging Contrast (PIC)-mapping, which displayed non-quantitative crystal orientation at the nanoscale as gray levels in ratios of images acquired at different linear polarizations [2]. A later development provided grayscale, semi-quantitative PIC-maps by acquiring stacks of 19 images as the linear polarization was rotated in 5° intervals from 0° to 90° [3-7]. The latest development uses the same stacks of images to fully, quantitatively display crystal orientations in colors, including hue and brightness, which represent in-plane and off-plane crystallographic c-axis orientation angles [8-10].

Using PIC-mapping in these 3 subsequent modes, we discovered several biomineral formation mechanisms in nacre [11,7], sea urchin teeth [12-14], ascidian spicules [10], corals, eggshells, modern and fossil sea shell ultrastructure [15].

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3:00pm **SA+AS+BI+MI-TuA3 New Dimensions in Synchrotron IR Spectroscopy**, ***Michael Martin***, Lawrence Berkeley National Laboratory **INVITED**

Synchrotron infrared beamlines use the diffraction-limited beam properties to enable a variety of cutting edge science - how can we go further?

By combining scattering-scanning near-field optical microscopy (s-SNOM) with mid-infrared synchrotron radiation, synchrotron infrared nano-spectroscopy (SINS) enables molecular and phonon vibrational spectroscopic imaging, with rapid spectral acquisition, spanning the full mid-infrared (500-5000 cm^{-1}) region with nanoscale spatial resolution. This highly powerful combination provides access to a qualitatively new form of nano-chemometric analysis with the investigation of nanoscale, mesoscale, and surface phenomena that were previously impossible to study with IR techniques. We have installed a SINS end-station at Beamline 5.4 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory, making the s-SNOM technique widely available to non-experts, such that it can be broadly applied to biological, surface chemistry, materials, or environmental science problems. We demonstrate the performance of synchrotron infrared nano-spectroscopy (SINS) on semiconductor, biomineral and protein nanostructures, providing vibrational chemical imaging with sub-zeptomole sensitivity.

The spatial field localization at the tip apex can also result in a large near-field momentum sufficient to optically excite phonon polaritons (PhPs), which are quasiparticles resulting from the strong coupling of photons with optical phonons. Here, we use SINS to image the PhP spectral response in thin hexagonal boron nitride (hBN) crystals. The large spectral bandwidth of the synchrotron source enables the simultaneous measurement of both the out-of-plane (780 cm-1) and in-plane (1370 cm-1) hBN phonon modes. In contrast to the strong and dispersive in-plane mode, the out-of-plane mode PhP response is weak. Measurements of the PhP wavelength reveal a proportional dependence on sample thickness for thin hBN flakes [2].

This talk will present the novel SINS instrumentation and a variety of scientific examples. Future directions, both technical and scientific, will be discussed.

*With Hans A Bechtel, Markus B. Raschke, Z. Shi, F. Wang, R.W. Johns, D.J. Miliron, E.A. Muller, R.L. Olmon

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4:20pm **SA+AS+BI+MI-TuA7 Sample Delivery Methods for X-ray Free Electron Lasers**, ***Uwe Weierstall***, Arizona State University **INVITED**

Serial crystallography at XFEL's has shown great promise in recent years for solving crystal structures of proteins, which produce only micron sized crystals. Liquid jets have been very successful for delivery of microcrystals to the X-ray beam. The commonly used liquid injection system will be discussed. High sample consumption has motivated the development of an injector, which uses high viscosity media like Lipidic Cubic Phase (LCP). G-protein coupled receptors are an important group of membrane proteins which are often crystallized in LCP. The injector generations a microscopic stream of LCP with adjustable speed for sample delivery to the X-ray beam[1]. Some important GPCR structures could be solved with this device at the LCLS[2]. In addition, new media with similar viscosity to LCP have been developed which enable delivery of soluble or membrane proteins into the X-ray beam with low sample consumption[3]. The high viscosity injection method has also been shown to facilitate serial diffraction experiments with microcrystals at synchrotron microfocus beamlines. This talk will highlight these developments and discuss the possibilities.

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5:00pm **SA+AS+BI+MI-TuA9 Synchrotron-based Spectroscopy Investigation for Electronic Phase Transition at Highly-Charged Electric-Double-Layer Interfaces**, ***Hongtao Yuan***, SLAC National Accelerator Laboratory **INVITED**

Electric-field control of charge carrier density has attracted much attention since it is remarkably simple for modulating physical properties of condensed matters and for exploring new functionalities with a transistor configuration. Owing to the limitation of dielectric breakdown in most solid dielectrics, the maximum carrier density accumulated in conventional field-effect transistors (FETs) is quite low (<< 10^{13} cm^{-2}) and thus seriously limits the tunability of electronic states of solids, for example, not sufficient enough to induce insulator-to-superconductor transition. While the electric-double-layer transistor (EDLT) with ionic liquids (ILs, or ionic gel) as gate dielectrics have been proved to be able to effectively attain a high carrier density up to levels of around 10^{15} cm^{-2} and to realize a large local electric field up to 50 MV/cm at liquid/solid interfaces. For example, electric-double-layer transistors have been demonstrated for an electric-field control of emergent interfacial quantum phenomena and the electronics phase transitions in condense matters, such as insulator-superconductivity and paramagnetism-ferromagnetism transitions. However, the mechanistic/spectropic understanding of the local electronic structures at such highly charged IL/oxide EDL interfaces and also further modification under gate-bias remain elucidated and challenging.

In this talk, we conducted synchrotron radiation based X-ray absorption spectroscopy (XAS) and Auger electron spectroscopy (AES) combined with in situ electrical measurements to directly characterize the evolution of the electronic structure at a representative IL/$La_{0.7}Sr_{0.3}MnO_3$ (LSMO) thin film interface. We find a significant valence reduction localized to the topmost LSMO layer after interface formation, and that the gate-bias predominantly modulates this surface reduced Mn species effectively converting these top layers into an insulator. We expect the synchrotron radiation based photon science probing techniques will directly shed light on the understanding of interfacial electronic phase control under the electric field.

(This work was done in collaboration with Bongju Kim, Jun-Sik Lee, Yasuyuki Hikita adn Harold Y. Hwang. This work was supported by the Department of Energy, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering, under contract DE-AC02-76SF00515.)

5:40pm **SA+AS+BI+MI-TuA11 Correlation of the Conductivity/Magnetic Properties and the Electronic, Crystalline and Compositional Structure of Strongly Correlated Complex-oxide Interfaces and Thin Films***, Juan Rubio-Zuazo,* SpLine CRG Beamline at the ESRF The European Synchrotron, France*; G.R. Castro,* SpLIne CRG Beamline at the ESRF The European Synchrotron, France

We study the structural and electronic properties of strongly correlated complex-oxide thin films and interfaces using Hard X-ray Photoelectron Spectroscopy (HAXPES), Electron Energy Loss Spectroscopy (EELS) and Grazing Incidence X-ray diffraction (GIXRD) at the BM25-SpLine beamline (Branch B) at the ESRF. Strongly correlated complex-oxide exhibit a wide variety of interesting physical properties which originate from mutual coupling among spin, charge and lattice degrees of freedom. Usually, the interface drives the magnetic and electric response of the heterostructure. The chemical, mechanical, electric and magnetic properties of such devices are often intimately related to the structure, composition profile and morphology of their surface and internal interfaces. Several mechanisms are present at these interfaces as crystallographic space group modification, presence of oxygen vacancies, dislocations due to lattice strain, deviation from stoichiometry, phase segregation. In general all these phenomena modify the intrinsic properties of the materials used at the heterostructure, offering a unique way to produce artificial correlated materials with tailored properties. The growth of these materials in thin film form opens possibilities for magneto-electronic and spintronic devices applications. The results shown here are focused on the study of the influence of buried interfaces on the electric and magnetic properties of CMR and multiferroics systems. We will show the experimental methodologies at SpLine based on synchrotron radiation techniques to gain quantitative knowledge on the crystallographic and electronic properties at the interface between different complex oxides. There are few techniques able to provide an accurate insight of what is happening at these buried interfaces which in general are buried by several tens of nanometres in the material. The simultaneous combination of hard and soft X-ray photoelectron spectroscopy, electron energy loss spectroscopy with surface/interface X-ray diffraction gives unique capabilities in this respect. Here we will present a series of example to show how the interface properties can change the magnetic-conductivity properties.

6:00pm **SA+AS+BI+MI-TuA12 Interface Passivation of III-V/High-k Materials by High Energy X-ray Photoelectron Spectroscopy: A Quantitative Evaluation***, Thierry Conard, V. Spampinato, L. Nyns, S. Sioncke,* IMEC, Belgium*; J.M. Ablett,* Synchrotron SOLEIL- Ligne GALAXIES, France*; W. Vandervorst,* IMEC, KU Leuven, Belgium

The use of InGaAs as a high carrier mobility CMOS-channel material requires a proper electrical passivation of its interface with the gate dielectric. One of the passivation schemes investigated involves the use of Sulphur. In this work, high-k stacks on Sulphur passivated InGaAs substrates involving both Al2O3 and HfO2 are investigated. A major question related to the use of Sulphur relates to the chemical states at the interfaces. XPS is traditionally an important technique for interface analysis but faces several challenges in its application to the above mentioned stacks. First, due to the large number of elements involved, numerous peak interferences are present limiting the choice of useful photoemission peaks. Second, relevant stacks have total thicknesses of the order of 4 nm, which lead to very low intensities , certainly for minority elements like Sulfur. In this work, we discuss the impact of the H_2S passivation temperature as well as the use of TMA pre-pulses in the growth of Al_2O_3. We show that the Sulphur bind to In but that no As-S or Ga-S bonds could be detected. The use of a TMA pre-pulse after surface passivation leads to a reduction of the amount of Sulphur present at the interface and likely increases the amount of In-O bonds. Higher temperature H_2S passivation leads to a reduction of the amount of Sulphur at the surface.

We also observe that the presence/absence of S at the interface, as well as the presence of the Al_2O_3 buffer, which has a major impact on the relative peak position in the spectra between the substrate and the overlayer. This will be compared with the electrical characteristics of the stacks.

Finally, we show that using the Sessa software, full quantification of the stack can be obtained under the condition that all instrumental parameters are correctly taken into account.

Biomaterial Interfaces
Room Hall D - Session BI+PB-TuP

Biomaterial Interfaces Poster Session (preceded by Oral Flash Presentations)

BI+PB-TuP2 Quantitative Sensing of Pancreatic Enzymes using Gelatin, ***George Banis,*** University of Maryland, College Park*; L. Beardslee,* Walter Reed National Military Medical Center*; R. Ghodssi,* University of Maryland, College Park

We present an investigation of gelatin film response to an array of pancreas-specific enzymes using a Quartz Crystal Microbalance (QCM) system. In fluids secreted from the pancreas into the upper small intestine, highly concentrated enzymes (α-amylase, trypsin, and lipase) are mixed to complete digestion of partially broken-down materials entering from the stomach. Sensors, such as that illustrated in Fig 1, are utilizing biomaterials such as gelatin, as it can be used to enter the body due to its biocompatibility and tailorability both chemically and structurally. Gelatin is known to be highly sensitive to degradation by trypsin, one of the aforementioned enzymatic pancreas biomarkers, thereby offering potential to indirectly monitor trypsin levels [1]. The composition of pancreatic fluid is a biomarker in testing exocrine function of the pancreas, a process often involving invasive procedures toward quantifying losses in enzyme levels [2]. However, its interactions with other interfering enzymes such as lipase or α-amylase have not been studied. While these enzymes are believed to cleave specific to bonds not found in gelatin, it is critical to be able to determine how these non-specific enzymes impact the signals produced when gelatin interacts with specific enzymes, i.e. trypsin, when they are each in the media.

In this work, we utilize a QCM system in sensing the mass change of gelatin films deposited onto standard crystals with gold electrodes. Films are subjected to a constant flow rate of buffer before introducing pancreatic enzymes in the setup illustrated in Fig 2. After system stabilization under buffer flow, material loss is quantified from the surface of the crystal. For trypsin, as expected, we observe degradation in a concentration-dependent manner, shown in Fig. 3. With either lipase or α-amylase, however, we observe no change, as illustrated in the top of Fig 4. After rinsing with buffer, we reintroduce trypsin in combination with each enzyme to determine if the presence of nonspecific enzymes affected the sensitivity of the gelatin to proteolytic activity, shown in the bottom of Fig 4. Digestion rates are found to decrease by -----83% and 77% with exposure to lipase or α-amylase, respectively, indicating a decrease in gelatin sensitivity to trypsin in the presence of these enzymes. The next phase in this work will be to combine all three enzymes to further model pancreatic juices. This work emphasizes the necessity in characterizing gelatin's response to other enzymes in understanding its sensitivity and specificity in the digestive environment, leading the avenue for devices designed to monitor gastrointestinal health.

BI+PB-TuP3 Evaluation of Printed Cell Viability, Proliferation, and Insulin Production on Various Alginate-Gelatin Hydrogels, ***Luis Solis,*** *J. Rincon, A. Varela-Ramirez, R. Aguilera, T. Boland,* University of Texas at El Paso

Over the past couple of decades, encapsulation of islets or beta cells has emerged as the new modality for the treatment of Type 1 Diabetes Mellitus (T1DM). A major setback in bioengineering encapsulated cells however, is the formation of fibrosis from immunologic defenses rendering the cells ineffective. This study proposes the use of an inkjet bioprinter to allow arrangement of βTC-6 mouse pancreatic beta cells and improve vascular ingrowth among alginate hydrogels. In addition, different concentrations of gelatin were tested in order to determine printable alginate-gelatin ratios for optimal vascular ingrowth, proliferation, viability, and insulin production of cells. Cell proliferation cultures were monitored daily for a total duration of 14 days. Cell viability and glucose stimulated insulin production were assessed at day 14. In-vitro alginate-gelatin hydrogels promoted proliferation of spherical insulinoma clusters and increased insulin secretion as compared to the monolayer of cells without hydrogels. These findings demonstrate that the alginate-gelatin hydrogels support the proliferation, viability, and insulin production of βTC-6 cells. These results will also allow to formulate improved bioinks for automated cell encapsulation applications.

BI+PB-TuP4 Synchrotron Radiation Studies of the Bonding and X-Ray Induced Reactions of Bacteriorhodopsin Adsorbed on Gold, ***Richard Rosenberg,*** Argonne National Laboratory*; D. Mishra, R. Naaman,* Weizmann Institute of Science, Israel

Bacteriorhodopsin (bR) is the integral protein of the purple membrane of *Halobacterium salinarum* and is the most studied proton pump. It is a chiral system composed of seven parallel, upright-oriented alpha-helices. Recent photoemission and electrochemical studies have shown that it can act as a natural electron spin filter as a result of the chiral-induced spin selectivity effect.[1] Previous structural studies using hard x-ray synchrotron radiation (SR) have shown that such radiation can significantly impact the structural integrity of bR,[2] while earlier work has demonstrated that X-ray-induced, low energy secondary electrons can play a major role in surface chemical reactions of adsorbed biological molecules.[3] In the present study we use SR x-ray absorption and photoelectron spectroscopy (XPS) to characterize the initial state of the adsorbed bR. Time-dependent changes in the core-level XPS spectra are utilized to follow the dynamics of the X-ray/secondary electron induced reaction. The results will be discussed in terms of previous studies of x-ray induced reactions in bR and other biological molecules.

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BI+PB-TuP5 Investigations on Peptide Incorporation and Peptide Yields in ME-SIMS, ***Martin Körsgen,*** *A. Pelster, M. Heeger, B.J. Tyler, K. Dreisewerd, H.F. Arlinghaus,* Universität Münster, Germany

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a powerful technique for the nanoanalysis of biological samples, but improvements in sensitivity are needed in order to detect large biomolecules, such as peptides, on the individual cell level at physiological concentrations. An increase in the detection efficiency for larger molecules and reduced fragmentation rates could be obtained by a) the use of cluster ion beams such as Au_n^+ , Bi_n^+ , C_{60}^+ , or even large Ar_n^+ clusters in order to maximize the energy deposited close to the surface or b) by modifying the surface by organic matrices in the so-called matrix-enhanced SIMS (ME-SIMS). This approach is based on embedding analyte molecules in low weight organic matrices, like common MALDI matrices, prior to ion bombardment

We used dual beam ToF-SIMS to image the incorporation of three peptides with different hydrophobicities, bradykinin, substance P, and vasopressin, into two classical MALDI matrices, 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid (HCCA) prepared with dried droplet sample preparation method. For depth profiling, an Ar cluster ion beam was used to gradually sputter through the matrix crystals without causing significant degradation of matrix or biomolecules. A pulsed Bi_3^+ ion cluster beam was used to image the lateral analyte distribution in the center of the sputter crater. Using this dual beam technique, the 3D distribution of the analytes and spatial segregation effects within the matrix crystals were imaged with sub-µm resolution.

Combining cluster ion beams and ME-SIMS we were able to investigate the molecular yield of two peptides (bradykinin and melittin) under various primary ions and preparation methods. Large argon clusters in the mass range between 1000 and 2500 atoms per cluster and several bismuth primary ions were used to determine molecular yields. Preparation utilized spin coating of pure peptide solutions and spray coating of matrix-peptide mixtures on silicon wafers. With the data obtained we were able to describe the molecular yield of the analyzed peptides. For bismuth primary ions the yield obtained by the use of cluster primary ions is nearly constant in the case of ME-SIMS, whereas for the neat sample an increase of the molecular yield is observable. In contrast to the molecular yield decrease with larger argon clusters for neat samples, an increase of the molecular yield is observable for larger argon clusters in the case of ME-SIMS.

BI+PB-TuP6 Developments of Non-Stick Surfaces for Medical Devices: Beneficial Effects of Thin Film Metallic Glass Coating, *G.H. Jiang, C.C. Yu, C.L. Li, Y. Tanatsugu,* ***Jinn P. Chu,*** National Taiwan University of Science and Technology, Taiwan, Republic of China*; M.J. Chen, S.H. Chang,* Mackay Memorial Hospital Tamsui Campus, Taiwan, Republic of China

This presentation reports on the use of Zr-based ($Zr_{53}Cu_{33}Al_9Ta_5$) thin film metallic glass (TFMG) for the coating of various medical devices and compares the results with those obtained using conventional titanium nitride and pure titanium coatings . TFMG was selected as the coating material for its unique properties such as good biocompatibility and antibacterial property due to its amorphous atomic structure, revealing a great potential for biomedical applications. The TFMG coating was shown to reduce insertion forces and retraction forces by up to over seventy percent when tested using polyurethane rubber block. The benefits of TFMG-coated needles were also seen when tested using pig muscle tissues. Based on the nano-scratch test, the TFMG coatings achieved a low coefficient of friction (COF), about one order of magnitude lower than those of bare surface and other coatings. Furthermore, the adhesions of cancer cells and platelets to coatings are also examined. TFMG coating is shown to appreciably minimize the attachment of cancer cells and platelets by more than eighty percent in relative to those of Ti coating and bare surface. The low COF and non-stick coated surfaces by TFMG can be attributed to the absence of grain boundaries in the TFMG coating, smooth surface and low surface free energy.

BI+PB-TuP7 Polyurethane Degradation by Wild Type and Hydrolase Deficient *Pseudomonas protegens* Pf-5 Unsaturated Biofilms, ***Daniel Barlow,*** US Naval Research Laboratory*; LJ. Nadeau, C.S. Hung,* Air Force Research Laboratory*; J.C. Biffinger,* US Naval Research Laboratory*; A.L. Crouch,* Air Force Research Laboratory*; J.N. Russell,* US Naval Research Laboratory*; W.J. Crookes-Goodson,* Air Force Research Laboratory

Two hydrolases secreted by *Pseudomonas protegens* Pf-5 bacteria, PueA and PueB, have been demonstrated to be active towards polyester - polyurethane (PU) hydrolysis. In this work, the impact of these enzymes towards PU degradation was directly compared at biofilm / PU interfaces through deletion of PueA and PueB genes. Unsaturated biofilm assays were used where biofilm growth took place on solid, hydrated PU discs in air. PU degradation was analyzed using confocal Raman microscopy of intact samples. Additionally, cross-sectional analysis of microtomed sections was done using FTIR microscopy and combined atomic force microscopy – infrared spectroscopy (AFM-IR). Results showed varying degrees of biofilm related permeation and polymer degradation within the ~300 um thick discs. Degradation took place through a pitting process involving preferential loss of the ester component. Wild type and PueB knockout mutants showed the highest levels of hydrolysis, measured by loss of carbonyl intensity in vibrational spectra, while the PueA and double knockout mutants showed lower hydrolysis levels. The apparent higher level of PueA activity is consistent with higher enzymatic activity for the hydrophobic lipase substrate, p-nitrophenyl palmitate. Relationships between biofilm morphology and PU degradation were also observed for the wild type and mutant biofilms.

BI+PB-TuP8 Laser Irradiation of Mg Alloys: Reduced Kinetics and Enhanced Biocompatibility, *M.A. Melia,* ***David Florian,*** *W. Steuer, J.R. Scully, J.M. Fitz-Gerald,* University of Virginia

Until recently, biodegradable implants have exclusively been used in non-load bearing applications such as stents and sutures. Mg-Al-Zn alloys like AZ31B are currently being considered as biodegradable materials because of their similar mechanical properties to that of bone. In addition, the corrosion product resulting from Mg alloys in body fluid contains Mg and Ca-phosphates (hydroxyapatite) and has been shown to stimulate bone regeneration. The degradation of Mg alloys is also considered non-toxic when corroding in the human body. However, the structural integrity is poor due to rapid corrosion caused by microstructural heterogeneities in the form of electrochemically noble secondary phases leading to micro-galvanic couples and preferential dissolution of the α-Mg matrix in physiological media. Laser surface processing of the Mg-Al-Zn alloy, AZ31B, reduced the corrosion rate in simulated body fluid (SBF) experiments by minimizing the impact of secondary phases.

Experiments utilized a pulsed excimer laser (λ = 248 nm and FWHM = 25 ns) in combination with a novel surface modification chamber. Samples of AZ31B in the as-received and laser processed condition were submerged in a 27 mM HCO_3^- Tris ($(HOCH_2)_3CNH_2$) variant of SBF. The corrosion resistance was investigated through optical microscopy, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), gravimetric mass loss, and polarization measurements.

No major corrosion product variations were observed for the as-received / laser processed specimens by SEM and EDS, both showing a similar amount of Ca and P. The laser processed alloy exhibited a reduction in anodic kinetics compared to the as-received material, suggesting the corrosion product is more compact and passivating. Furthermore, the laser processed surface exhibited a 50% reduction in mass loss after 24 hours immersion in SBF in comparison to the as-received samples. Optical micrographs of samples immersed in SBF reveal a reduction in the H_2 evolution rate of the laser processed versus as-received material. In addition, the laser treated specimens exhibited a significant increase in wettability with a 10^o contact angle compared to the 45^o angle of the as-received materials. The increased wettability of the laser processed samples may decrease the time required for osseointegration through allowing cells to more readily bind to the surface of an implant.

BI+PB-TuP9 Plasma-assisted Fabrication of Silver/Bacterial Cellulose/Chitosan Functional Nano-composites and Their Properties, ***Shuquan Chang,*** *A.R. Shetty, S.L. Arias Suarez, J.P. Allain,* University of Illinois at Urbana-Champaign

Bacterial cellulose and chitosan are renewable natural polymers and have many favorable properties such as biocompatibility, biodegradability and low toxicity. So, they have been extensively used in drug delivery systems, gene therapy, tissue engineering, and biosensor applications. Silver nanoparticles have attracted much attention for their unusual chemical and physical properties and have been widely applied in sensors, antibacterial and photocatalytic areas. The synthesis of nanomaterials with different chemical composition, size distribution, and controlled monodispersity has become an important research area in nanotechnology. Many kinds of methods such as vapor deposition, solvent-thermal, sol-gel, electrochemistry and microwave have been developed to fabricate nanomaterials. So far, plasma technology has become an important approach to prepare and reinforce materials and surfaces. This work seeks to fabricate nanoparticles/natural polymer functional composites via an atmospheric pressure plasma method. Comparing many traditional methods, atmospheric pressure plasma jet can induce chemical reactions in mild conditions, which can guarantee the purity of system and will not destroy the structure of natural polymers.

In this work, silver/bacterial cellulose/chitosan functional composites are fabricated via an atmospheric pressure plasma method. Plasma can produce many active radicals including reduction species and oxygen species, which can trigger chemical reaction. Ag+ in the reaction system can attach to the surface of bacterial cellulose and chitosan via bonding. By controlling the reaction condition, Ag+ can be reduced to Ag(0) and form Ag nanoparticles under plasma treatment. The existence of bacterial cellulose and chitosan can limit the growth and prevent the aggregation of particles, which is very critical to form nanostructure. SEM, XRD, XPS, FT-IR are employed to examine the morphology and structures of as prepared nano-composites. The biocompatibility and antibacterial properties are also studied. All results reveal that Ag nanoparticles are successfully formed and well dispersed in bacterial cellulose/chitosan. The as prepared silver/bacterial cellulose/chitosan nano-composites have excellent biocompatibility and antibacterial abilities, which can be used in biomedical areas. This convenient synthesis strategy based on atmospheric pressure plasma could be extended to fabricate other nanoparticles/bacterial cellulose/chitosan composites.

BI+PB-TuP11 A Non-toxic, Super-Hydrophilic Anti-Fog Coating for Lenses used in Closed Body Cavity Surgery: VitreOx TM– In Vivo Animal Clinical Trials, ***Nicole Herbots,*** SiO2 NanoTech LLC*; C.F. Watson,* SiO2 NanoTech LLC/Arizona State University Physics Dpt*; EJ. Culbertson,* University of California at Los Angeles*; PR. Thilmany, IPO. Martins,* SiO2 NanoTech LLC

Laparoscopes, arthroscopes, and laryngoscopes lenses are hydrophobic and fog during closed body surgery, due to bodily fluids and differences between body and operating room temperatures [1,2]. Surgeons must repeatedly remove, clean, and reinsert scopes obscured by fog. Hencesurgery duration, infection risks, and scarring from air exposure increase. Methods to address fogging introduce other complications [3]. Alcohol-based coatings scar tissue and quickly evaporate, heated lenses require reheating every 5 to 20 minutes. A non-toxic, super-hydrophilic, anti-fog coating that is pH neutral (7.2-7.4), long-lasting has been developed VitreOx™ . [4] VitreOx™ can be used wet or dry, without alcohol, heat, or fluid evacuation. When applied in liquid form, it easily espouses a lens's surface and edges, and dries within seconds to form a permanently

super-hydrophilic surface on silica and most polymer surfaces . VitreOx™ avoids current shortfalls by foregoing frequent reapplications.

VitreOx™'s anti - fog properties can be explained by nucleation and growth theory, and the three mechanisms for condensation: 1) 3-D droplets, resulting in fogging; 2) 2-D sheets resulting in a flat transparent film . ; or 3) mixed, resulting in optical distortion. On hydrophobic surfaces (e.g. optical lenses), condensation occurs with fogging via spherical 3-D droplets (Volmer-Weber model). 3 - D droplets scatter light in all directions through refraction yielding opaque or translucent films, or fog. VitreOx™ applied to hydrophobic lenses renders them super-hydrophilic. Similar to the 2-D Frank Van-der-Merwe growth mode, ***condensation with uniform wetting*** yields transparent 2-D films that don't distort light.

In-vitro and *in-vivo* animal studies of VitreOx™ were conducted to measure performance and duration of anti-fog effectiveness and bio-compatibility. *In-vitro* testing spanned from 3 to 72 hours over a 3-year range. Side-by-side *in-vivo* gastro-endoscopies were conducted using a lens coated with VitreOx™ and a Covidien Clearify ™ warmer with anti-fog, on Yucatan™ swine for 90 minutes . The VitreOx™ coating lasted without fogging nor reapplication, while Covidien Clearify™ only l ast ed at for 38 minutes without fogging, and required retreatment and reapplication . No adverse reaction was observed on swine in surgery, and in the 18 months that follows.

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Biomaterial Interfaces
Room 101A - Session BI+MI-WeM

Biosensors and Diagnostics

Moderators: Daniel Graham, University of Washington, Tobias Weidner, Max Planck Institute for Polymer Research, Germany

8:00am **BI+MI-WeM1 Bacteriophage-Derived Surfaces for the Targeting of Pathogenic Bacteria*, Stephane Evoy,*** University of Alberta, Canada

Bacterial pathogens cause a high level of morbidity and mortality, specifically for infants, young children, elderly and immunocompromised individuals. Antibodies have been exploited as molecular probes in order to impart specificity to bacterial biosensing platforms. Antibodies however suffer from degradation and reliability issues. The high specificity of phages offers a potent alternative for the targetting of pathogens. More specifically, recombinant phage receptor binding proteins (RBPs) responsible for phage-host specificity can be used as biological probes and present numerous advantages over the use of whole phage.

We successfully coupled phage RBP Gp047 from phage NCTC12673 onto magnetic beads. These beads were then employed for the extraction of Campylobacter cells from food matrices. Recovery rates were greater than 80% in samples spiked with as low as 10^2 cfu mL^{-1} of cells. Phage lysins have also been employed as capturing probes. We coupled recombinant lysin Gp10 from the mycobacteriophage L5 onto magnetic Dynabeads 280 for the capture of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) cells from complex media. The study employed skim cow milk spiked with MAP cells, skim milk spiked with both MAP and *Escherichia coli* cells and Middlebrook 7H9 medium spiked with MAP cells as test matrices. The functionalized beads were incubated with the spiked sample, separated, cleaned, and subjected to DNA extraction. The resulting solution was analyzed by real time PCR. The entire test was completed within 24 hours. The capture process significantly increased the PCR sensitivity and demonstrated high specificity towards MAP cells.

Further, we demonstrated the use of cysteine-tagged P22 phage RBPs on gold surface for the specific SPR detection of *Salmonella enterica serovar Typhimurium*. These results demonstrate that N-teminus Cys tagged proteins capture bacteria efficiently compared to the C-terminus Cys tagged protein due to preferential orientations. Finally, micromechanical devices have also been proposed for the detection and enumeration of bacteria. We designed and developed a microresonator array optimized for such detection. This large array-based design offers a large total area for the capture of cells, while maintaining the ability to detect the attachment of a single cell anywhere on the array. The devices were functionalized with phage GST-Gp48 tail-spike proteins to impart specificity of detection. We successfully employed these arrays for the specific detection of *Campylobacter jejuni* from clean buffer. The devices did not show any sensitivity to *Escherichia coli* bacteria confirming the specificity of detection .

8:20am **BI+MI-WeM2 Biomolecule Sensing at Attogram Levels via Nanophotonic-Optomechanical Resonators*, Anandram Venkatasubramanian,*** University of Alberta, Canada*; V.T.K. Sauer, S.K. Roy,* National Institute of Nanotechnology, Canada*; D. Wishart, W.K. Hiebert,* University of Alberta, Canada

The Gas chromatography (GC) – Mass spectrometry (MS) system is the industry benchmark in chemical analysis. However the need for complex instrument such as the ionizer makes the Mass spectrometry unsuitable for portable detection applications. Recent demonstrations with nano-optomechanical (NOMS) resonators at atmospheric pressure show they are promising for portable GCs, matching the mass detection limits of NEMS sensors in only the first generation. Owing to their superior displacement sensitivity compared to NEMS, NOMS may have competitive advantages going forward. In this regard, a free space interferometry system was used for NOMS sensing of biomolecules. The primary motivation to develop sensors for portable applications is to develop point of care diagnostic devices for health monitoring. As the state of our health is a product of our interactions with our environment, metabolomics is useful in health monitoring. Among the different human biofluids, urine is “favoured” due to their precise potrayal of metabolic breakdown products, sterility and easy to obtain large volumes. Hence we have demonstrated multiple component (5 +) biomolecule detection from derivatized human urine metabolomes (HUM) as they elute from the GC. Derivatized HUMs such as ethyl malonic acid (EMA) were tested as a single component sample to obtain the limit of detection. From the results it was observed that the minimum detectable mass was about 20 attograms with a concentration threshold of 25 µM with EMA, which is in the normal physiological range in human adults. T o the best knowledge of the authors, this is the first time a NOMS based gas sensor has been used in conjunction with a gas chromatographic system and has demonstrated physiological range of detection of biomolecules.

8:40am **BI+MI-WeM3 Hole-mask Colloidal Lithography Method to Fabricate Chiral Metal-Nanoparticles for Plasmon Enhanced CD Measurements*, Gunnar Klös,*** Aarhus University, Denmark*; D.S. Sutherland,* Aarhus, Denmark

Hole-mask colloidal lithography is a well studied method [1] for a reliable high throughput fabrication of metal nanoparticles (NP). The plasmonic resonances and their electromagnetic near field dependencies of such NPs are widely used as bio-sensors , e. g. for high sensitivity refractive index sensing [2]. Rather recently it was also shown that the near field interactions of planar plasmonic chiral NPs can be used for sensitive chiroptical measurements of very dilute amounts of chiral material [3], allowing structural characterization of even small amounts of many biomolecules. So far the fabrication of such chiral NPs is based on time-consuming techniques such as e-beam lithography [4].

Here, I present a novel method for the fabrication of chiral Au nanocrescents based on a modified version of hole-mask colloidal lithography. This reliable and efficient method utilizes the shrinking of the hole due to the material evaporated through it, adding an additional parameter to the control over the shape of the resulting NP.

The method allows the fabrication of nanocrescents with an outer diameter of 100nm-200nm that show plasmonic responses similar to previous Au structures [2]. Furthermore, when analyzed with circular polarized light, they show a considerable circular dichroism response.

Hence, this fabrication method is a promising technique for the time- and cost-efficient production of sensitive biosensors for the structural analysis of chiral materials.

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9:00am **BI+MI-WeM4 Neuraminidase Assay using Glycan-Functionalized Graphene Field-Effect Transistors*, Kaho Kamada,*** *T. Ono, Y. Kanai,* Osaka University, Japan*; Y. Ohno,* Tokushima University, Japan*; K. Maehashi,* Tokyo University of Agriculture and Technology, Japan*; K. Inoue,* Osaka University, Japan*; Y. Watanabe,* Kyoto Prefectural University of Medicine, Japan*; T. Kawahara, Y. Suzuki,* Chubu University, Japan*; S. Nakakita,* Kagawa University, Japan*; K. Matsumoto,* Osaka University, Japan

A lot of the anti-influenza virus drugs such as Tamiflu® and Relenza® prevent the viruses from infecting to the next cell. Influenza viruses enter the host cells of the throat and trachea by binding to the host cell’s surface receptor molecules which contains sialic acid. After the proliferation into the cell, the viruses cleave the sialo oligosaccharides by the action of the enzyme neuraminidase (NA), and propagated viruses are detached from the cells on the infection to the next cell. Therefore, it is possible to suppress the chain of propagation of virus by inhibiting the NA. Currently, the evaluation of antiviral drugs has been conducted mainly using cultured cells, there are problems in accuracy and quantitative property. In addition, it is difficult to evaluate the mechanism of reaction. Therefore we aim to build a useful new biological model platform for drug evaluation and drug discovery research. We modified sialoglycoprotein chain on the graphene surface, and fabricated the glycan-functionalized Graphene Field-Effect Transistors (G-FET), which reproduce cell surface environment on the graphene. The reaction behavior of the virus is highly detected as the current by the G-FET. So we can quantitatively evaluate drug reaction by the physical indicators. As a first step here, we electrically measured NA reaction by the glycan-functionalized G-FET.

G-FET was produced by evaporating the electrodes on graphene obtained by the exfoliation method. 1-Pirenbutan acid succinimidyl ester as a linker, was modified human sialoglycoprotein chain having a modified amino group on the graphene channel. After dropping the NA on it, we measured time course of the neuraminidase reaction monitored by the graphene-FET.

When dropping the NA, the current value is decreased exponentially. This is because the sialic acid negatively charged was disconnected from the

sugar chain, and the hole carriers induced on graphene were decreased. The rate of decrease in current value with neuraminidase dropping is in good agreement with the activity value obtained by the absorption method (A NA molecule cuts the 1.7 of molecules per second). This shows that the rate constant obtained from electrically measurement by G-FET reflects the enzyme reaction rate.

This study has received the support of JST・CREST.

9:20am **BI+MI-WeM5 Surface-sensitive Imaging of Supported Membranes and Single Lipid Vesicles for Medical Applications***, **Fredrik Höök,*** Chalmers University of Technology, Sweden **INVITED**

Measurements of ligand-binding events to membrane-protein receptors in a near-natural environment display an opportunity in mechanistic studies of membrane receptors. Furthermore, the residence time of drug-target interactions is being increasingly recognized as a key parameter in evaluating drug efficacy, but is hampered by the technical challenge to perform such studies for membrane proteins. However, with membrane proteins embedded in nanoscale lipid vesicles and detection methods with single molecule sensitivity, such information can be gained in a broad dynamic range, as requested in both drug-screening and diagnostic applications. A diverse set of tools with single-nanoparticle sensitivity is now available, to which we recently contributed a concept that enables simultaneous fluorescent and scattering-based label-free imaging of thousands of surface-bound nanoscale entities [Agnarsson B et al., ACS Nano, 2015]. The principle is based on the use of lipid vesicles as enhancer elements in optical waveguide based fluorescence and label-free evanescent-wave scattering microscopy, making the concept compatible with analysis of both water-soluble and cell-membrane bound receptors. The concept is currently evaluated as a diagnostic assay for biomarker detection and in drug-screening applications, previously explored by us using conventional total internal reflection fluorescence (TIRF) microscopy [Gunnarsson et al., Anal Chem (2015)]. The use of scattering microscopy in the context of single-enzyme detection in complex biological fluids will be presented, with focus on single-molecule biomarker detection in cerebrospinal fluid from individuals suffering from Alzheimer's disease [Angew Chemie, 2015]. A new means to utilize the two-dimensional fluidity of supported cell-membrane derived lipid bilayers in microfluidic designs for nanoparticle size determination and sorting applications will also be presented [Simonnson et al., JACS, 2011 and Pace et al., Anal Chem, 2015].

11:20am **BI+MI-WeM11 Non-invasive Thermal Sensing using Thermographic Phosphors***, **Firouzeh Sabri,*** University of Memphis*; S. Allison,* EMCO*; P. Parajuli,* University of Memphis

F. Sabri[1], S. W. Allison[2], and P. Parajuli[1]

1. University of Memphis, Department of Physics and Materials Science

2. Emerging Measurements Co.

Thermal measurements involving thermographic phosphors, whether in the form of powder, crystal, or glass, continues to be of interest for a wide range of applications and temperature ranges. The investigation of phosphor-doped polymer films is a promising avenue for thermometry applications. Phosphor thermometry has been investigated recently for non invasive thermal assessment of biological and biomedical surfaces. For thermographic phosphors to be useful for biomedical applications they must first be encapsulated in a biocompatible, biostable, and transparent "host" that would allow optical access to the embedded phosphors. The work here demonstrates the feasibility of thin film thermographic phosphor-based thermometry where La_2O_2S:Eu particles have been embedded in a clear silicone encapsulant at different concentrations. The composite materials were prepared by means of spin-coating technology and the effect of spin speed and spin time on the thickness and distribution of the powder was investigated. . In order to improve the thermal conductivity of the composite material, a layer of carbon has been incorporated into the multilayer structure. The results presented will compare the excitation-emission behavior of the composite materials mentioned above with the properties of pure powder, at various temperatures. The effect of the tensile properties of the composite material on the excitation/ emission behavior of the materials will also be discussed. Measurements were conducted at low temperatures and at elevated temperatures and the decay characteristics were investigated as a function of temperature.

11:40am **BI+MI-WeM12 Imaging Time-of-Flight Secondary Ion Mass Spectrometry to Characterize Tumor Progression and Regression***, **Lara Gamble,*** *B.M. Bluestein, D.J. Graham,* University of Washington*; F. Morrish, D. Hockenbery,* Fred Hutchinson Cancer Research Center

The tumor microenvironment has been associated with regulating tumor cell growth, metastatic potential, and chemotherapeutic drug resistance. However, very few techniques are capable of directly probing the tumor microenvironment on the micron scale. A new perspective is required to interpret and characterize this complex environment. Using imaging time-of-flight secondary ion mass spectrometry (ToF-SIMS) and a mouse model with Myc-dependent inducible and regressible pancreatic β-cell neoplasia, it is possible to relate changes in the composition and distribution of metabolic related molecules with tumor development. Myc, one of the most frequently deregulated oncogenes in human cancers, contributes to tumorigenesis through various mechanisms, including the deregulation of cell proliferation and growth, protein and mitochondrial biogenesis, and metabolic alterations. Pancreatic tissues were harvested and frozen in optimal cutting temperature (OCT) at 6 days post Myc induction. 4 µm cryosections were serially cut, with one used for H&E staining, one for ToF-SIMS analysis, and another for immunohistochemistry. ToF-SIMS data was acquired on an IONTOF TOF V with pulsed 25 keV Bi_3^+ ion beam. Principal component analysis (PCA) of ToF-SIMS image data separated regions of tumor cells from stroma within the first principal component and revealed subtle differences in chemistry between the tumor and surrounding tissue. ToF-SIMS data suggests a preferential uptake of fatty acids 18:3, and 18:2 within the tumor. The tumor also shows an increased localization of sphingomyelin fragments and vitamin E compared to the surrounding tissue. PCA was also applied to selected tumor regions to spatially and chemically analyze within the tumor and compare chemistries between different tumor sizes. Distinct chemical differences were identified between control and 6 day Myc activated β-cell islet tissues using multivariate analysis techniques. The results from C14:0 and phosphatidylcholine fragments present within the tumor are suggestive of *de novo* fatty acid synthesis. This work further demonstrates the high resolution capability of ToF-SIMS as the data clearly reveals intra-tumor chemical heterogeneity as localized high intensity regions, but histologic correlations are needed to discern the purpose and function of these structures.

12:00pm **BI+MI-WeM13 $SrI_2(Eu^{2+})$Gamma Camera for SPECT Imaging in Medical Applications***, **LaNell Williams,*** *M. Groza, E. Rowe, J. Butler,* Fisk University*; T. Peterson,* Vanderbilt University*; A. Burger,* Fisk University

The detection of gamma rays for nuclear imaging has become increasingly important in designing non-invasive imaging tools for biological research and modeling. Although imaging techniques such as computed tomography (CT) and Positron Emission Tomography (PET) have been previously used, and improved spatial resolution and sensitivity continue to be an issue. Thus, improvements in these detection devices are needed to create better images for more accurate modeling in research [Cressey, 2011]. Scintillators such as Cesium Iodide (CsI), and Sodium Iodide (NaI) have been used for many imaging techniques for their ease of growth, energy resolution, and overall effectiveness as a gamma ray detectors. In more recent studies, Strontium Iodide doped with Europium ($SrI_2(Eu^{2+})$) has shown to be a promising scintillator compared to NaI and CsI. Because of's $SrI_2(Eu^{2+})$ improved energy resolution (~2.7%), fast decay time (~1.2 µs) and light yield (110,000 photons/MeV), it is an ideal replacement for technologies that have used previously been made with NaI and CsI. [Cherepy, 2008]. In addition, $SrI_2(Eu^{2+})$ also has an emission centered around 420 nm making it an ideal scintillator to be used with silicon photomultipliers that provide lower energy consumption than the standard photomultiplier tube. The improved energy resolution of $SrI_2(Eu^{2+})$ in a gamma camera will result in an promising detector for nuclear imaging.

Biomaterial Interfaces
Room 101A - Session BI+AS+SA-ThM

Synthesis and Processing of Biomaterials/Biologically Inspired Materials

Moderators: Daniel Barlow, US Naval Research Laboratory, Lara Gamble, University of Washington

8:00am **BI+AS+SA-ThM1 Response of PC 12 Cells to Mesoporous Substrates with and without DC Bias**, *F. Sabri,* University of Memphis*; **Kyle Lynch**,* Universtiy of Memphis*; O. Skalli,* University of Memphis

The interaction of nerve cells with nanostructured surfaces and substrates is of great importance to the field of tissue engineering and artificial substrates developed for biomedical applications. It has been established that cells respond to different polymer surface characteristics such as roughness, surface free energy, topography, chemistry, charge, and other properties including electrical conductivity. It has also been recognized that the nanotopography can affect and influence cell morphology, cell alignment, cell signaling and extension of neurites. Here, we discuss the influence of the mesoporous structure of crosslinked silica aerogels on the adhesion, proliferation, and neurite extension of PC 12 cells, in the presence and absence of applied DC bias. The behavior of cultured PC 12cells on the aerogel substrates is compared to the behavior of cells cultured on cell culture plastic (control) and the affect of applied DC bias of different magnitudes is carefully investigated. The neurite extensions clearly show a preferred growth direction and the rate of growth of extensions is also influenced by the varying conditions.

8:20am **BI+AS+SA-ThM2 Collagen Functionalized with ALD-TiO_2: A Novel Biomaterial for Bone Grafting**, ***ArghyaKamal Bishal***, *C. Sukotjo, C.G. Takoudis,* University of Illinois at Chicago

In medicine, the use of implants is growing rapidly. Some patients may not have enough bone to support such implants.[1, 2] Therefore, those patients are required to have augmentation, a procedure to increase the height or width of inserted bone-like supporting materials, prior to implantation.[1] Collagen resorbable membrane is used as a bone grafting material which acts as supporting material and facilitates new bone formation.[3] Sometimes, titanium reinforced collagen membrane is used for improved stability.[2]

Collagen is an important biomaterial which is used in several biomedical applications. It has a triple helix structure made of polypeptide chains.[3, 4] Hydrogen bonds play an important role in keeping together these peptide chains. Glycine, proline are the most abundant amino acids found in its structure. Collagen has also the ability to be reorganized and crosslinked and thus turn into flexible fibrils with higher tensile strength.[3]There are four main types of collagen: type I, II, III and V. Among them mostly type I and little amount of type V construct the bone structure by forming a composite with hydroxyapatite (HA) crystals.[4]

Titania (TiO_2) itself is biocompatible.[5] Additionally, it has the ability to attract Calcium and Phosphate in a liquid environment.[6] Therefore TiO_2 coated collagen may be used as an excellent bone grafting material to nucleate Ca and P and thus reconstructing a stable bone structure. In this work, we present ALD of TiO_2 on collagen membrane in a custom-made ALD reactor. The deposition was performed at room temperature. Tetrakis(dimethylamido)titanium (TDMAT) and ozone were used as metal precursor and oxidizer, respectively. Samples were characterized for their surface morphology, composition and mechanical properties. Energy dispersive spectroscopy confirmed the presence of Ti on coated collagen and electron microscopy showed an increase in fiber diameter after deposition by more than a factor of 2:

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8:40am **BI+AS+SA-ThM3 Nanostructure Formation on Biomaterials by Directed Irradiation Synthesis (DIS) for Tissue Regeneration and Maximize Corrosion Resistance**, ***Jean Paul Allain***, *A.R. Shetty,* University of Illinois at Urbana-Champaign*; S. Arias, A. Barnwell,* University of Illinois at Urbana Champaign*; F. Echeverria, L.F. Berrio,* University of Antioquia, Colombia

An important aspect of tissue engineering is to create a favorable extracellular microenvironment, mainly the extracellular matrix (ECM) which can guide cell differentiation and tissue regeneration. The ECM consists of a number of cues that can be guided by surface topography and matrix stiffness [1]. Recent studies [2,3] have demonstrated that depending on the type of surface structuring and patterning, cell adhesion can be controlled with potential applications in smart cell culture systems and biosensors. Many of the desired biomaterial properties that require a combination of metal alloy and soft material interfaces cannot be processed with conventional bottom-up techniques. Directed irradiation synthesis (DIS) address this limitation by introducing a synthesis process that is scalable to high-volume manufacturing by virtue of its intrinsic large-area simultaneous exposure of materials surfaces and interfaces.

In this study, we have employed directed irradiation synthesis to induce nanostructure formation on two commonly used biomaterials: 1) Ti_6Al_4V and 2) magnesium (Mg). The goal is to examine the role of surface nanostructuring on the stimulation of cells and tissues in order to provide important cues for tissue regeneration as well as guarantee a good corrosion resistance and to minimize bacteria adhesion. Detailed characterization, establishing processing conditions and correlating them to surface and biomaterial properties have been successfully performed on nanostructured medical grade Ti_6Al_4V and Mg. These irradiated surfaces were biologically evaluated by using human aortic smooth muscle cells (HASMCs) for cytotoxicity and cell/surface adhesion and interactions. This analysis allowed us to determine connections with processing, structure, surface energy, and biointerface properties. Biological response of these new surfaces has also lead us, for the first time, to establish correlations between nanostructuring by DIS and cell stimulation, as well as to show the real potential of these new surfaces to favorably stimulate cells and tissues different than bone. The corrosion behavior of these biomaterials in a phosphate buffered simulated body fluid (SBF) has also been investigated for bone implant application.

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9:00am **BI+AS+SA-ThM4 Controlled Peptide Surfaces of Various Ratios that Guide Neural Stem Cell Differentiation**, ***HalaShakib Dhowre***, *M. Zelzer, H. Sahaf, C. Towlson, N.A. Russell,* University of Nottingham, UK

Cell instructive biointerfaces represent an essential aspect for the advancement of regenerative medicine. Currently, a major issue in biointerface design is the limited ability to mimic the complex interactions of the natural processes in the extracellular matrix (ECM) with artificially designed surfaces and interfaces [1]. While biomaterial surfaces have been shown to be able to elicit specific cell responses (e.g. adhesion, proliferation, differentiation), precise control akin to that of natural cellular environments is still lacking[2].

AIM:

The present work aims to address this challenge by designing new synthetic peptide surfaces with well controlled composition and functionality able to impact control over the differentiation of neuronal stem cells with the ultimate goal to understand and control how neuronal networks function.

METHODS:

Compositionally well defined surface concentrations of two short laminin peptide sequences, Arg-Gly-Asp (RGD) and Ile-Lys-Val-Ala-Val (IKVAV) were prepared of various ratios via the “grafting from” stepwise approach and the surface modification was confirmed with surface analysis techniques to

indicate successful peptide functionalisation. The neural stem and progenitor cells (NSPC) were set up from embryonic rat hippocampi (E18). Immunocytochemistry (ICC) observed cell viability and differentiation to specific NSPC lineages for Nestin, βIII-Tubulin and GFAP.

RESULTS:

Surface characterising techniques (WCA, AFM and ToF-SIMS) verified the successful amino acid build-up to peptides on the surfaces, allowing modification of the surfaces with RGD and IKVAV. Enhanced NSPC adhesion, proliferation and differentiation were observed on the peptide surfaces. ICC demonstrated Nestin expression decrease after the removal of the growth factors (EGF and FGF) and an increase in the expression of βIII-Tubulin and GFAP; thus illustrating cells differentiating from stem cells to neurons or astrocytes due to peptide surface influence.

CONCLUSION:

Well defined peptide surfaces were designed successfully, the various ratios of RGD and IKVAV surfaces demonstrated cell adhesion, proliferation and i desirable effects in controlling different populations of stem cell fate. These surfaces may advance new insight in understanding how surface properties affect the regulation of physiological relevance in directing neural cell differentiation, which will be essential to understand how neural networks function.

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9:20am **BI+AS+SA-ThM5 Biofunctional Hydrogels for Tissue Repair***, **Andres Garcia,*** Georgia Institute of Technology **INVITED**

Hydrogels, highly hydrated cross-linked polymer networks, have emerged as powerful synthetic analogs of extracellular matrices for basic cell studies as well as promising biomaterials for regenerative medicine applications. A critical advantage of these synthetic matrices over natural networks is that bioactive functionalities, such as cell adhesive sequences and growth factors, can be incorporated in precise densities while the substrate mechanical properties are independently controlled. We have engineered poly(ethylene glycol) [PEG]-maleimide hydrogels to study epithelial morphogenesis and identified independent contributions of biophysical and biochemical properties of these materials to this developmental process. In another application, we have developed synthetic hydrogels that support improved pancreatic islet engraftment, vascularization and function in diabetic models. These studies establish these biofunctional hydrogels as promising platforms for basic science studies and biomaterial carriers for cell delivery, engraftment and enhanced tissue repair.

11:00am **BI+AS+SA-ThM10 Nanoscale Domain Formation Induced by Partial Polymerization Creates Planar Supported Lipid Bilayers that are Fluid and Stable***, **N.Malithi Fonseka**, B. Liang, K.S. Orosz, C.A. Aspinwall, S.S. Saavedra,* University of Arizona

Planar supported lipid bilayers (PSLBs) are widely explored bilayer platforms for receptor-based biosensors. PSLBs composed of fluid lipids lack the stability necessary for many technological applications due to the relatively weak non-covalent interactions between lipid molecules. Lipid polymerization enhances bilayer stability, but may greatly reduces lipid mobility and membrane fluidity. In an effort to enhance bilayer stability while maintaining fluidity, we have prepared and characterized PSLBs composed of mixtures of the polymerizable lipid bis-Sorbyl phosphatidylcholine (bis-SorbPC), and the fluid lipid diphytanoyl phosphatidylcholine (DPhPC) to form mixed PSLBs. We measured lateral diffusion coefficients (*D*) as a function of the bis-SorbPC/DPhPC molar ratio using fluorescence recovery after photobleaching (FRAP). In pure DPhPC PSLBs, D = 0.66 μm^2/sec. In equimolar poly(bis-SorbPC)/DPhPC, D = 0.36 μm^2/sec, whereas when the ratio is greater than 0.7, D decreased to 0.13 μm^2/sec. These data show that considerable fluidity is retained even when the poly(bis-SorbPC) fraction is substantial, which suggests that these bilayers are phase segregated, composed of polymerized and fluid domains. However domains were not observed with fluorescence microscopy techniques. The sub-µm morphology of these PSLBs was therefore investigated using atomic force microscopy (AFM). Nano-scale phase segregation of the two lipids was observed. DPhPC forms a continuous lipid matrix that is 0.2-0.4 nm thicker than the island-like poly(bis-SorbPC) domains. This height difference agrees with bilayer thicknesses measured for pure DPhPC and poly(bis-SorbPC) PSLBs. Furthermore, it was observed that the size of the poly(bis-SorbPC) domains increased with the percentage of poly(bis-SorbPC) in the PSLB. In summary, mixed lipid bilayers composed of poly(bis-SorbPC) and DPhPC form nano-structured membranes with retained lipid diffusivity, and thus they have considerable potential for creating membrane-based biosensors in which receptor activity depends on bilayer fluidity.

11:20am **BI+AS+SA-ThM11 Stabilization of Lipid Films by Hyaluronic Acid and Polymeric Substitutes in a Joint Model System***, **Felicitas Schwoerer,*** Universität Heidelberg, Germany*; M. Trapp, R. Steitz,* Helmholtz-Zentrum Berlin für Materialien und Energie GmbH*; R. Dahint,* Universität Heidelberg

In the United States there are 27 million people suffering from osteoarthritis. The disease is primarily caused by the degeneration of cartilage, which covers the bone ends of the joints and is in turn decorated with a phospholipid (PL) layer. The bone ends are separated by the synovial fluid containing the polysaccharide hyaluronic acid (HA) as a main component. It is generally assumed that both HA and PLs reduce friction and protect the cartilage. Based on the observation that HA concentration is reduced in diseased joints, a new cure called viscosupplementation has been developed, where HA or mixtures of HA and PLs are injected into the joints. However, until now the positive effect of such therapy is under debate.

To elucidate the importance of HA and PLs for joint lubrication and protection on a molecular level we investigate their interaction using a simplified model system for natural joints. A silicon wafer (representing the bone end) is covered with PL oligobilayers and incubated in an aqueous solution containing HA or polymeric substitutes (representing the synovial fluid). To mimic the forces in joint movement, we expose the model surfaces to a home-built shear apparatus facilitating *in situ* measurements at a rotational speed between 0 rpm and 6000 rpm. Measurements were performed at *BioRef* (Helmholtz-Zentrum Berlin), a time-of-flight neutron reflectometer with integrated infrared spectroscopy.

Upon contact with both HA and poly(allylamine hydrochloride) (PAH) solutions a tremendous swelling of the lipid film occurs. Film thickness increases by a factor of about four compared to pure D_2O exposure due to a drastic increase in the thickness of the interstitial water layers located between adjacent lipid bilayers. This effect is most likely due to the adsorption of charged polymers at the lipid headgroups leading to electrostatic repulsion. Despite their high film thickness and water content, the polymer-exposed lipid films exhibit approximately ten times higher shear stability than the respective systems incubated in pure water. With increasing rotational speed the lipid films contain substantially enhanced water fractions, which we attribute to increasing lateral fragmentation. Present investigations aim at the question whether HA and PAH are incorporated into the lipid tail region and bridge adjacent bilayers as this might explain the observed higher stability.

11:40am **BI+AS+SA-ThM12 New Substrates and Patterning Methods for Supported Lipid Bilayers***, **Sally McArthur,*** *L. Askew,* Swinburne University of Technology, Australia **INVITED**

The cell membrane encases and protects cellular components and plays an important role in transport, signalling and disease. Studying membrane behaviour is a challenging task due to the complexity and scale on which these processes occur. Supported lipid bilayers (SLBs) have provided researchers with stable and reproducible platforms to recreate cell membrane environments. The planar structure of the model means a variety of patterning techniques can be employed to recreate membrane architecture on both a micro and nanoscale. In particular, pre-patterned substrates are of great interest as they eliminate complications associated with preserving membrane integrity during patterning. Plasma polymers provide a versatile method of creating thin films with a variety of different surface chemistries. In this work we explore the behaviour of plasma coatings in aqueous conditions and the use of plasma films for creating patterned SLBs using vesicle collapse. The results demonstrate that variations in plasma polymer chemistry can be used to control lipid bilayer formation and the locations of different lipid species. Characterisation of film behaviour and bilayer formation was conducted using a variety of techniques including ellipsometry, quartz crystal microbalance with dissipation (QCM-D), confocal microscopy, atomic force microscopy (AFM) and Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS).

MEMS and NEMS

Room 102B - Session MN+BI-ThM

'Fantastic Voyage' – the New Micro/Nano/Bio Systems Frontiers

Moderators: Reza Ghodssi, University of Maryland, College Park, Christian Zorman, Case Western Reserve University

8:00am **MN+BI-ThM1 Living Micromachines**, ***M. Taher Saif***, *B. Williams,* University of Illinois at Urbana Champaign **INVITED**

Industrial revolution of the 19th century marked the onset of the era of machines that transformed societies. Late 20ttthe century marked the beginning of miniaturization resulting in micro-nano electronics and MEMS/NEMS. This revolution connected every individual with all the others in the planet. However, all of these machines are non-living, and they do not have inherent intelligence. On the other hand, since the discovery of genes, there is a considerable body of knowledge on engineering living cells. It is thus appropriate to envision biohybrid micro machines that are made from microfabricated scaffolds and living cells. These machines have the potential of unprecedented capabilities, as they would carry the footprints of millions of years of evolution. These machines may emerge from an interaction between the living cells and the micro-nano scaffolds. Thus, they might be the unique products of both the bottom-up and top-down methods. In this talk we will present such an elementary micro machine consisting of a soft slender string and rat cardiomyocytes. The string is made from PDMS by filling a microfabricated channel using capillary draw. Cells are cultured on one region of the string. These cells interact with the string as well as with each other, and beat in synchrony as a single actuator. This living actuator bends the string, and a bending wave propagates from the actuator site towards the end, as a bending of a sperm. This artificial machine thus swims in fluids as the engineered living swimmer. These swimmers might be used in vivo for autonomous intelligent drug delivery.

8:40am **MN+BI-ThM3 Inertial Imaging with Nanoelectromechanical Systems**, ***Selim Hanay***, Bilkent University, Turkey

Nanoelectromechanical Systems (NEMS) resonators can be used as exquisite sensors of physical quantities, such as mass, force and spin. As the size of a mechanical sensor shrinks, its responsivity increases: combining this advantage with low-noise readout schemes has enabled extreme sensitivity levels to be achieved such as the detection of electronic spins, single-cells in liquid and volatile chemicals at low concentration. It has become possible to use NEMS sensors for single-molecule analysis: for instance, the mass of single molecules can be measured by NEMS, paving the road for sensitive biochemical analysis. Another sensing modality, for single-biomolecule analysis, has been recently discovered. It was shown that spatial properties of analyte particles, such as its size and degree of asymmetry, can be extracted by tracking multiple mechanical modes of a sensor. Such multimodal mesaurement provides both size and shape information, as well as, the mass of the analyte. Furthermore, by combining spatial information, an image of the analyte can be constructed. The new technique, Inertial Imaging, transforms the capabilities of nanomechanical sensors to a new level: the combined data of molecular mass, size and shape of the analyte can enable previously unattainable information in biomolecular analytics. In this talk details for the implementation of this technique as well as experimental progress for single molecule detection will be presented.

9:00am **MN+BI-ThM4 Dynamic Patterning of Breast Cancer Cells Using Silicon Nitride Multimode Membrane Resonators**, ***Hao Jia****, *H. Tang, P.X.-L. Feng,* Case Western Reserve University

Manipulating and patterning biological cells on surfaces has gained great interest due to its fundamental importance for cell-level biophysical studies, and implications for potential biomedical applications.[1] In this work, we report the first experimental demonstration of non-invasive, fast, dynamic patterning of clusters of cancer cells with micrometer-scale spatial precision, by using multimode silicon nitride (Si_3N_4) membrane resonators that feature very large aspect ratios (~600nm-thick, and hundreds of microns in lateral dimensions), in rectangular and square shapes. The fabricated Si_3N_4 membranes exhibit robust multiple flexural resonances (within 50–500 kHz) in liquid solutions. We observe that the breast cancer cells (MDA-MB-231) can be dynamically manipulated into diverse Chladni patterns[2] within a few seconds, via the multimode resonances of the membrane. Multiple spatially signatory cell patterns are observed for rectangles (~300×120μm^2) and squares (~350×350μm^2), respectively. We further demonstrate that cell patterns can be dynamically switched. We model and explain the cell patterns by oscillating boundary induced acoustic steaming flows of the fluid.

As an important cell line for breast cancer metastasis studies, MDA-MB-231 cells are selected in this work and genetically-engineered to express green florescent protein (GFP), a biomarker for gene expression and cell identification. Dilution in EDTA solution allows suspended individual cells with reduced surface viscosity. Cancer cells are locally delivered to the device area using micropipettes, and the cell distributions are imaged in real time by a high-speed fluorescent microscope. We observe that cancer cells are allocated into '1D' array of clusters on the rectangular membrane under excitation frequencies corresponding to its 1st, 2nd and 3rd modes (in Fig. 1), while they cluster into multiple '2D' patterns when the multiple modes of square membrane are excited individually (in Fig. 2). Furthermore, the cell patterns can be readily changed when switching between resonance frequencies.

The demonstrated Si_3N_4 membrane platform provides new capabilities for manipulating breast cancer cells, which could further lead to studies involving cancer cell signaling and interaction with neighboring cells, probing and controlling cancer cell metastatic behaviors using multimode mechanical resonators.

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9:20am **MN+BI-ThM5 Smart Drug Delivery through Gut**, *W. Yu, R. Rahimi, M. Ochoa,* ***Babak Ziaie***, Purdue University **INVITED**

This paper reports on a magnetic-proximity-fuse method for site-specific drug release in the gut. As an embodiment, a capsule is comprised of two compartments; one houses a charged capacitor and a reed switch (magnetic switch) while the other accommodates the drug reservoir, whose lid is latched by a taut nylon thread intertwined with a NiCr wire. Meanwhile, the wire is connected to the capacitor via the switch. Once within the proximity of a magnet, the switch closes and the capacitor discharges on the wire, melting the fusible thread and opening the reservoir.

Pharmaceutical companies have been interested in releasing medications at specific sites in the gut. Many drug absorption studies (DASs) use Enterion [1] capsule which incorporates a loaded spring that is RF-actuated, pushes a piston and forces the drug out through a hole. The capsule location is monitored via γ-ray scintillating-imaging. Other similar devices include InteliSite [2] (tracked by x-ray) and IntelliCap (tracked by pH). Though suitable for DASs in clinical settings, these approaches are not practical for use in larger populations. This is mainly due to the problems related to the need for real-time localizing the capsule. Thus, we present an alternative approach using a Smart Capsule incorporating a magnetic-proximity fuse.

Fig. 1 depicts a capsule traveling through the gut in proximity to a magnet. The capsule consists of two chambers. The right one contains a capacitor (1F, 2.7V) and a reed switch. The capacitor is charged before use. A NiCr wire is connected to the capacitor and the switch, with two ends in the right chamber and the rest intertwined with a nylon fuse in the drug reservoir. Along the axis is a rubber band linking the fuse to a cap, covering the reservoir with an elastic layer for reliable sealing. Additionally, an elastic rod is used as a loaded spring pushing the cap out once the fuse breaks. As in Fig. 2, the band holds the cap initially. When the capsule meets a magnetic field to close the switch, the NiCr wire is heated, melting the fuse (60~85ºC) and opening the cap. Fig. 3 shows a photo of a Smart Capsule (9mm×26mm).

Fig. 4 shows snapshots of the capsule traveling in a tubing at various points with respect to the magnetic marker. The capsule starts to open in frame "a" (1sec) at a separation distance of 1mm from the magnet, with the opening completed (cap totally separated from the capsule) in frame "e" (17sec). Considerable diffusion and mixing of the powdered dye in water is observed within a minute, frame "f". Fig. 5 shows the relationship between the capacitance and the charging voltage necessary for melting the fuse through a NiCr wire (~0.23ohms).

11:00am **MN+BI-ThM10 Biopsy with Untethered Microgrippers**, ***David Gracias***, Johns Hopkins University **INVITED**

An important requirement to enable the vision of the Fantastic Voyage is to develop tiny mechanized devices that can be used to perform functional surgical tasks. As an example, I will describe a decade long effort in our laboratory to develop sub-millimeter sized biopsy forceps in the shape of

* **MEMS/NEMS Best Paper Award Finalist**

hands or microgrippers. I will discuss challenges in fabrication, materials choice, deployment, guidance, safety, power harvesting and practical surgical operation of these devices. In addition to in vitro and ex vivo studies, I will discuss in vivo operation of such devices in live pigs to enable statistical sampling of tissues, targeted biopsy and drug delivery. I will also discuss the creation of microgrippers out of bioresorbable and biodegradable silicon based and polymer materials as well as devices as small as single red blood cells. Our studies highlight feasibility and proof-of-concept for the implementation of small untethered devices in medicine and surgery.

11:40am **MN+BI-ThM12 Bacterial Biofilms on 3D-printed Implant Materials**, ***Ryan Huiszoon***[*], *S. Subramanian, T.E. Winkler*[†], University of Maryland, College Park*; H.O. Sintim,* Purdue University*; W.E. Bentley, R. Ghodssi,* University of Maryland, College Park

New technologies, like capsule microsystems (Fig.1), have the potential to revolutionize medical care by autonomously locating and treating *in vivo* infections. Such implantable systems require 3D structures that cannot be fabricated using traditional photolithography. Additive manufacturing is the ideal tool that allows for rapid and detailed fabrication of such complex structures. However, 3D-printed implants, like their metal and ceramic counterparts, are vulnerable to biofilm infections [1]. Thus, it is essential to characterize treatments for these films on emerging 3D printable materials. In this work, we evaluate bacterial biofilm treatment on 3D-printed implant materials such as MED610.

Bacterial biofilms are the leading cause of implant infections. They form when planktonic bacteria adhere on a surface, secrete protective extracellular matrix and grow. They are highly resistant to antibiotics, allowing the infection to persist [2,3]. Microfluidics is a common and effective tool to evaluate biofilms in a controlled environment as it offers more clinically relevant data about biofilm growth and treatment [4].

In this work, we 3D printed open microfluidic channels (750µm wide x 400µm deep) on a Stratsys Connex3 polyjet printer. The open channels were sealed using sealing wrap, PDMS and glass, and clamped together using binder clips (Fig.2). *Escherichia coli* W3110 biofilms were grown in the micro-channels under Lysogeny Broth (LB) media flow at 120µl/hr for 24 hours. Subsequently, treatment with LB media (control), 10µg/mL gentamicin, 100µM autoinducer-2 (AI-2) analog (quorum sensing disruptor), or a combination of the latter two treatments was introduced into the various channels at the same flow rate for an additional 24 hours. The biofilms were then imaged through the open channels using an Olympus BX60 microscope (Fig.3). Treatment efficacy was evaluated as a percent change in channel surface coverage quantified using ImageJ. Bacterial biofilm coverage was reduced by 21%, 31% and 50% for gentamicin, analog and combination treatments respectively as compared to the untreated control, consistent with previous results (Fig.4) [2]. The combination treatment proved most effective, reducing biofilm coverage by 37% compared to the standard antibiotic-only therapy [2].

3D printed microfluidic test platforms offer an affordable way to experiment on new materials and can hasten the development of novel treatments. Additionally, the characterization of these materials brings us a step closer to making this technology a viable option for fabricating complex structures for implantable multi-purpose microsystems.

[*] **MEMS/NEMS Best Paper Award Finalist**
[†] **National Student Award Finalist**

Spectroscopic Ellipsometry Focus Topic
Room 104C - Session EL+AS+BI+EM+TF-ThA

Optical Characterization of Nanostructures and Metamaterials (2:20-3:40 pm)/Application of Spectroscopic Ellipsometry for the Characterization of Thin Films (4:00-6:00 pm) and Biological Materials Interfaces

Moderators: Tino Hofmann, University of North Carolina at Charlotte, Stefan Zollner, New Mexico State University, Heidemarie Schmidt, Technische Universität Chemnitz, Germany

2:20pm **EL+AS+BI+EM+TF-ThA1 Optical Properties of (Self-assembled) Nanostructured Surfaces Studied by Spectroscopic Mueller Matrix Ellipsometry and Local Direct Imaging Techniques**, ***Morten Kildemo***, Norwegian University of Science and Technology, Norway **INVITED**

This paper covers several applications of ex-situ and in-situ Spectroscopic Mueller Matrix Ellipsometry (SMME) for the study of self-assembled nanostructured surfaces, with applications ranging from antireflection coatings, PV-absorbers, nanoimprinting masks, plasmonic polarizers, plasmonic meta-materials and in particular hyperbolic metamaterials and meta-surfaces. The optical analysis is systematically supported by AFM, SEM and TEM. As nanostructured surfaces are often inherently anisotropic, SMME with variable angle of incidence and full azimuthal rotation of the sample is shown to be a powerful optical technique to fully characterize such anisotropic and sometimes bi-anisotropic materials. The first part of the presentation briefly reviews an uniaxial effective medium approach to model the kinetics of the optical response of self-assembled straight and tilted GaSb nanopillars [Le Roy et al., Phys. Rev. B 2010, Nerbo et al. Appl. Phys. Lett. 2009], and SiO_2–nanopillars containing plasmonic Cu [Ghadyani et al., Opt. Exp. 2013]. The second part of the presentation discusses the experimentally extracted uniaxial and biaxial optical properties of self-assembled plasmonic hyperbolic meta-materials [X. Wang et al., *Block-copolymer based self-assembled hyperbolic metamaterials in the visible range.* (manuscript in preparation), 2016] and metasurfaces [Aas et al., Opt. Expr. 2013]. Hyperbolic metamaterials use the concept of controlling the propagative modes through the engineering of the dispersion relation, and are considered highly promising to reach different meta-properties. The presentation is closed by the discussion of the fascinating Mueller matrix response of a highly organized array of hemispherical Au nanoparticles produced by Focused-Ion-Beam milling, and the response is discussed in the context of highly organized meta-surfaces and plasmonic photonic crystals [Brakstad et al. Opt. Express 2015]

3:00pm **EL+AS+BI+EM+TF-ThA3 Optical Properties of Nanocrystalline Si_3N_4:TiN Thin Films**, ***Neil Murphy***, Air Force Research Laboratory; *L. Sun,* General Dynamics Information Technology; *J.G. Jones,* Air Force Research Laboratory; *J.T. Grant,* Azimuth Corporation

Nanocomposite films comprised of mixed nitrides, especially Si-Me-N (Me=Ti, Zr, Hf), have generated significant attention due to their robust thermal and mechanical properties. In addition to their desirable structural characteristics, the mixing of dielectric Si_3N_4 with various metallic nitrides has the potential for the deposition of hybrid thin films with controllable optical absorption based on the fraction of metallic nitrides present within the Si_3N_4 matrix. In this work, nanocrystalline Si_3N_{-4} thin films, doped with varying amounts of TiN (1-20 at.%), are deposited using reactive magnetron co-deposition. Note that the Berg model for reactive sputtering is utilized to select the initial conditions for the deposition of the films, which are sputtered from elemental targets within a mixed nitrogen-argon environment and characterized *in-situ* using spectroscopic ellipsometry. The TiN content is varied through systematic adjustment of the current applied to the Ti cathode concurrent with pulsed DC deposition of Si_3N_4 at a constant current of 0.4 A. The use of -*in-situ*- ellipsometry, interrogating wavelengths from 381-1700 nm, allows for the real-time measurement of the refractive index, extinction coefficient, and thickness of the growing films. Additionally, *in-situ* ellipsometry data is used to observe the behavior of the films at the onset of growth, indicating the onset of Volmer-weber type nucleation. All ellipsometric data are fit using a Bruggeman effective medium approximation, varying the amount of TiN present within the films. Optical characterization of the Si_3N_4:TiN thin films indicates that the refractive index at 550 nm decreases gradually from 2.05 to 1.99 as the TiN content is increased from 0-20 at%, while the extinction coefficient rises from 0 to 0.35. These films demonstrate strong absorption features starting from 550 nm out to 1500 nm, allowing for efficient absorption of visible and near-infrared wavelengths. Variation of the TiN content within Si_3N_4:TiN films allows for the user to select the magnitude of extinction coefficient and refractive index, leading to potential applications as mechanically robust layers in interference filters, or as alternatives to lossy metallic configurations in plasmonic devices.

3:20pm **EL+AS+BI+EM+TF-ThA4 The Effect of Aluminum Content on Properties of Al-doped Zinc Oxide Thin Films Grown at Room Temperature**, ***Lirong Sun***, General Dynamics Information Technology; *N.R. Murphy,* Air Force Research Laboratory; *J.T. Grant,* Azimuth Corporation; *J.G. Jones,* Air Force Research Laboratory

Transparent conductive Al-doped zinc oxide (AZO) thin films have shown excellent structural, optical and electrical properties for applications in photovoltaic and optoelectronic devices, transparent conducting electrodes, solar cells, liquid crystal displays, touchscreens, energy efficient window coatings and heat reflective coatings. In this work, the AZO thin films were deposited at room temperature by multi-target reactive magnetron sputtering using metallic Zn and Al targets simultaneously. The Al doping content of the AZO films by x-ray photoelectron spectroscopy (XPS) had great impacts on optical properties in the near infrared (NIR) and in the UV regions and were strongly correlated to their electrical properties. The spectroscopic ellipsometry data in three incident angles and transmission intensity data were measured and fitted simultaneously with a Tauc-Lorentz oscillator and a Drude model in the wavelength of 270 -2500 nm. The transmittance and reflectance spectra, the derived refractive index and extinction coefficient, were tailored in the NIR region by Al content and correlated to the electrical resistivity. The blue shift of the absorption edge in the UV region and the widening of the optical band gap were associated with the increase of the Al content. Structural, optical and electrical properties were characterized using x-ray diffraction, scanning electronic microscopy, UV-Vis-NIR spectra and four-point probe methods.

4:00pm **EL+AS+BI+EM+TF-ThA6 Optical Monitoring of Growth (and Death) of Thin Film Materials for Solar Cells**, ***Nikolas Podraza***, *K. Ghimire, M.M. Junda, A.A. Ibdah, P. Koirala,* University of Toledo; *S. Marsillac,* Old Dominion University; *R.W. Collins, Y. Yan,* University of Toledo **INVITED**

Performance of thin film solar cells depends on (i) electronic quality of the components (doped and undoped semiconductors, metallic and transparent conducting contact layers), (ii) component optical response, and (iii) full opto-electronic response of the photovoltaic (PV) device structure dictated by layer properties and thickness. Spectroscopic ellipsometry probes (ii) and (iii) through measurement of both thickness and optical response ($N = n + ik$, $\varepsilon = \varepsilon_1 + i\varepsilon_2$, $\alpha = 4\pi k/\lambda$) of multiple layers in thin film device structures. Assessing (i) electronic quality of materials or devices optically relies on understanding other property information deduced from the optical response, such as connecting variations in film structure (crystallinity, degree of disorder) or growth evolution to device performance. In situ, real time spectroscopic ellipsometry (RTSE) monitors growth evolution and post-deposition processes to better understand property changes with thickness, phase transitions and separation, and process kinetics. RTSE of hydrogenated silicon (Si:H), cadmium telluride (CdTe), and copper indium gallium diselenide (CIGS) absorbers have been used to understand growth and its relationship to the respective device performance. All of these are relatively mature PV technologies, where knowledge gained from RTSE during growth can potentially improve metrology and manufacturing. The potential impact of RTSE is equally strong when applied to developing technologies. Organometal lead halide perovskite semiconductors ($CH_3NH_3PbI_3$) are used in >20% initial efficiency solar cells but suffer from degradation with temperature, bias, moisture, and ultraviolet light exposure. The time scale of device performance degradation is much shorter than that of other polycrystalline PV (CdTe, CIGS). RTSE has been applied during co-evaporation of CH_3NH_3I and PbI_2 to produce the perovskite, but also during decomposition of the perovskite. Significant fractions of CH_3NH_3I and PbI_2 at the substrate / perovskite and perovskite / ambient interfaces after deposition even under simple atmospheric exposure begin to appear in a matter of minutes. The ability to track the degradation – or death of this material – in addition to growth may be equally important to assessing the ultimate stability and manufacturability of these next generation PV materials.

4:40pm **EL+AS+BI+EM+TF-ThA8 Monitoring Nanometer-Thin Film Formation using Ellipsometry**, ***Bert Müller***, *F.M. Weiss, T. Töpper, B. Osmani,* University of Basel, Switzerland

Elastomers can transform electrical energy into mechanical one. They have a wide variety of appli-cations including powering wipers, sound

generation, and operating camera lenses. Sandwiched between electrodes the deformable but incompressible elastomer laterally expands when apply-ing a voltage. To provide the necessary strain of at least 10 %, micrometer-thick silicone mem-branes need an operation voltage of several hundred volts, which is inappropriate for the human body. Nanometer-thin membranes, however, require only a few volts. To generate forces as nec-essary for artificial sphincters, i.e. muscles to treat incontinence, several ten thousand membranes have to be sandwiched. Currently, the manufacturing methods such as organic molecular deposition only reach deposition rates of about one micrometer per hour, which does not allow fabricating the sandwiched nanostructures in an efficient way. We have developed an alternative deposition method to pre-pare extremely flat silicone membranes that are below one micrometer thick. The root-mean-square-roughness is smaller than one nanometer. For this purpose, silicone polymers in solution are sprayed by electrospray deposition [1,2]. Usually electrospraying is based on direct current mode. Here, we have employed, however, an alternating current to avoid charge accumulation on the substrate. Spectroscopic ellipsometry has been used to monitor the formation of confluent organic films and electrodes as well as the changes of the organic thin films during ultra-violet radiation treatments. This in situ technique enabled us to derive the refractive index, the porosity, the surface rough-ness, and the film thickness. The derived quantities on surface roughness and film thickness were validated using atomic force microscopy. The combination of electrospraying, ultra-violet light curing, and in situ ellipsometry has a huge potential to efficiently create and monitor nanometer-thin, ultra-flat elastomeric membranes, which may become part of artificial muscles for medical applications and beyond.

[1] F.M. Weiss, T. Töpper, B. Osmani, S. Peters, G. Kovacs, and B. Müller Electrospraying Nanometer-Thin Elastomer Films for Low-Voltage Dielectric Actuators Advanced Electronic Materials (2016) 1500476; DOI: 10.1002/aelm.201500476

[2] F.M. Weiss, T. Töpper, B. Osmani, H. Deyhle, G. Kovacs, and B. Müller Thin Film Formation and Morphology of Electro-sprayed Polydimethylsiloxane Langmuir 32 (2016) 3276-3283

5:00pm **EL+AS+BI+EM+TF-ThA9 Optical Determination of Electrical Response for Thin Film Transparent Conductors: Spectral Range Dependence**, ***Prakash Uprety***, *M.M. Junda, K. Lambright, R. Khanal, A. Phillips, M. Heben, D. Giolando, N.J. Podraza,* University of Toledo

Thin films with simultaneous high transparency and electrical conductivity have applications in photovoltaics, displays, and other opto-electronic devices. Accurate characterization of electrical transport properties along with optical properties in these transparent conductors, particularly when in the device structure, is of critical importance to their use. Spectroscopic ellipsometry (SE) provides a widely applicable method for determining such properties without many of the complications and limitations that accompany other methods that make use of physical contact to the film. As is described by the Drude model, free carrier optical absorption has increasing effect on the complex dielectric function ($\varepsilon = \varepsilon_1 + i\varepsilon_2$) with decreasing photon energies. Thus, extracting ε from SE measurements spanning the visible to terahertz (THz) frequency ranges provides sensitivity to film thickness and morphology at higher energies and free carrier absorption dominating the optical response at low energies. In this work fluorine doped tin oxide (SnO_2:F), aluminum doped zinc oxide (ZnO:Al), and sprayed single walled carbon nanotube (CNT) thin films are measured with ex situ SE over a spectral range of 0.035 to 5.9 eV using a single rotating compensator multichannel ellipsometer (0.75 - 5.9 eV) and a single rotating compensator Fourier transform infrared ellipsometer (0.035 - 0.75 eV). Additionally, the ZnO:Al and CNT films are measured using a single rotating compensator THz ellipsometer (0.4 - 5.8 meV) to further extend the measured spectral range to lower energies. Due to the wide spectral range measured, a single model describing ε and layer thicknesses has sufficient sensitivity to simultaneously determine electronic transitions, vibrational phonon modes, and free carrier absorption. The electrical properties in the Drude model are described by the bulk material resistivity ρ and scattering time τ. Optically extracted ρ has increasing correspondence to ρ deduced from four point probe electrical measurements as increasing low photon energies are included in the fitting (< 5% variation in ρ for ZnO:Al analzying the full measured range); a behavior that demonstrates the benefit of extending the measurement spectrum to very low energies. The analyzed spectral range dependence of optically determined transport properties in these examples is considered to illustrate how narrower spectral range measurements impact deduced ρ and τ.

5:20pm **EL+AS+BI+EM+TF-ThA10 Spectroscopic Ellipsometry Studies of CdS-CdSe-CdTe Alloys: Applications in Thin Film Solar Cells**, ***Maxwell Junda***, *C.R. Grice, Y. Yan, N.J. Podraza,* University of Toledo

Recent studies have demonstrated that photovoltaic (PV) device performance of thin film cadmium telluride (CdTe) solar cells is improved when a thin cadmium selenide (CdSe) layer is added at the cadmium sulfide (CdS) / CdTe interface and when oxygen is added to the CdS window layer (CdS:O). Specifically, devices fabricated with this configuration show increased short circuit current density without a corresponding degradation in open circuit voltage. The high temperature close spaced sublimation (CSS) deposition of the CdTe layers in these devices effectively anneals the existing CdS:O / CdSe window layer creating alloyed regions between these three materials as opposed to distinct, separate layers at the front side of the device. To better understand the sources of performance gain, we begin by using ex situ spectroscopic ellipsometry (SE) from the near infrared to the ultraviolet (0.74 – 5.9 eV) to study the optical and structural properties of these alloys. Films of CdS:O, CdS_xSe_{1-x}, and $CdSe_yTe_{1-y}$ are fabricated on soda lime glass substrates by radio frequency sputtering a stack of layered combinations of CdS, CdSe, and/or CdTe followed either by annealing at the CdTe CSS deposition temperature or actual CSS of CdTe. A parameterized model describing the critical point transitions in the optical response ($\varepsilon = \varepsilon_1 + i\varepsilon_2$) is developed, allowing for tracking of the changes in ε as a result of film composition and processing for each alloy. Additionally, structural and compositional variations introduced by the alloying of materials is considered and supported by complementary x-ray diffraction and energy dispersive x-ray spectroscopy measurements. The database of ε developed for these materials can be used to assess how the oxygen introduced in the CdS:O layer and diffusion of CdSe into both CdTe and CdS:O modify that interface and impact PV device performance.

5:40pm **EL+AS+BI+EM+TF-ThA11 Development of Growth Evolution Diagrams for RF Sputtered Nanocrystalline Hydrogenated Silicon Thin Films via Real Time Spectroscopic Ellipsometry**, ***Dipendra Adhikari***, *M. M. Junda, N. J. Podraza,* University of Toledo

As a result of its increased visible light absorption and increased stability in comparison to hydrogenated amorphous silicon (a-Si:H), hydrogenated nanocrystalline silicon (nc-Si:H) thin films are of considerable interest for a variety of opto-electronic applications, including photovoltaic (PV) devices. Radio frequency (RF) sputtering in an Ar + H_2 ambient provides a cost effective deposition technique for Si:H films and has advantages over conventional plasma enhanced chemical vapor deposition as a result of the potential to improve deposition rates and the elimination of hazardous precursor gasses. In this work we investigate how pressure, RF power, and Ar/H_2 ambient gas composition ratio influence film structure (thicknesses; amorphous, nanocrystalline, mixed phase composition) and optical response of Si:H films deposited by RF sputtering onto native oxide covered crystalline silicon wafer substrates using in situ real time spectroscopic ellipsometry (RTSE) over the near infrared to ultraviolet spectral range. Through analysis of RTSE measurements and application of virtual interface analysis where appropriate, the time evolution of bulk layer thickness, surface roughness, and complex dielectric function ($\varepsilon = \varepsilon_1 + i\varepsilon_2$) spectra are extracted. Variations in nucleation and evolution of crystallites forming from the amorphous phase as a function of pressure, power, or Ar/H_2 ratio can be deduced from the growth evolution and used to create growth evolution diagrams. Overall film quality, crystallinity, and hydrogen incorporation (assessed using infrared extended measurements), are also determined from ε. X-ray diffraction measurements provide complementary information about how deposition conditions influence the density, size, and preferred orientation of crystallites. In addition to controlling film phase and structure, improvement of the deposition rate is also of practical interest and is explored here.

Nanometer-scale Science and Technology
Room 101D - Session NS+BI-ThA

Applied Nanoscale Microscopy Techniques/Biomaterial Interfaces – New Advances

Moderators: Stephanie Allen, The University of Nottingham, UK, Leonidas Ocola, Argonne National Laboratory

2:40pm **NS+BI-ThA2 Advancing the Development of Nanocrystal Emitters *via* Advanced Electron Microscopy Techniques*, James McBride**, K.R. Reid, S.J. Rosenthal,* Vanderbilt University

The key tool for the characterization of nanoparticles has long been transmission electron microscopy. This technique can provide the size, shape, crystal structure and chemical composition of a nanocrystal. Aberration-corrected Z-STEM has enabled the visualization of the true core/shell structure of colloidal quantum dots, accelerating their commercial development.[1] Through dynamic STEM movies we have visualized the beam-induced motion of the surface atoms of nanocrystals and learned about the instability of the atomic structure of ultrasmall nanocrystals and the surface/sub-surface of large nanocrystals.[2] However, Z-contrast can be difficult to directly interpret due to the choice in shell material or uncertainty of the 3D morphology of large, thick-shelled quantum dots. Advancements in the detector design for performing STEM energy dispersive spectroscopy mapping (STEM-EDS) have greatly facilitated the chemical imaging of nanocrystals, enabling rapid identification of their chemical structure before significant beam damage occurs. With this technological advance, we have obtained the chemical composition of an individual nanocrystal and directly correlated to its individual photophysics using our recently developed correlation technique.[3] The unique combination of optical, structural and chemical information allowed us to determine the origin of the low quantum yield plaguing non-blinking CdSe/CdS quantum dots.[4] Further, STEM-EDS imaging will be presented showing development of InP/CdS and Zn_3N_2 nanocrystals. Included in the presentation will be specifics on sample preparation and the choice of beam current/spatial resolution and sample damage.

1. McBride, J.; Treadway, J.; Feldman, L.C.; Pennycook, S.J.; Rosenthal, S.J. Structural Basis for Near Unity Quantum Yield Core/Shell Nanocrystals *Nano Lett.***2006,** *6* (7), 1496-1501.

2. McBride, J.R.; Pennycook, T.J.; Pennycook, S.J.; Rosenthal, S.J. The Possibility and Implications of Dynamic Nanoparticle Surfaces *ACS Nano***2013,** *7* (10), 8358-8365.

3. Orfield, N.J.; McBride, J.R.; Keene, J.D.; Davis, L.M.; Rosenthal, S.J. Correlation of Atomic Structure and Photoluminescence of the Same Quantum Dot: Pinpointing Surface and Internal Defects That Inhibit Photoluminescence *ACS Nano***2015,** *9* (1), 831-839.

4. Orfield, N.J.; McBride, J.R.; Wang, F.; Buck, M.R.; Keene, J.D.; Reid, K.R.; Htoon, H.; Hollingsworth, J.A.; Rosenthal, S.J. Quantum Yield Heterogeneity among Single Nonblinking Quantum Dots Revealed by Atomic Structure-Quantum Optics Correlation *ACS Nano***2016,** *10* (2), 1960-1968.

3:00pm **NS+BI-ThA3 Demonstration of Electron Mirror for Quantum Electron Microscopy*, Navid Abedzadeh**, C.S. Kim, R.G. Hobbs, K.K. Berggren,* MIT

Electron mirrors have been used in electron microscopy techniques such as low-energy electron microscopy, mirror-corrected scanning electron microscopy and photoemission electron microscopy due to their ability to introduce chromatic and spherical aberrations of arbitrary sign. More recently, a design for a quantum electron microscope (QEM), an imaging approach based on interaction-free measurement, was proposed that could take advantage of an electron mirror whose surface was patterned with a topographic grating. This grating would produce a periodically varying potential close to its surface when a voltage was applied. As a result, the grating would diffract an incident electron plane wave, presenting an opportunity to develop a low-loss electron beam splitter. The diffracted beams produced by such a beam splitter could be used to probe a sample within an electron cavity to achieve an interaction-free measurement. An electron cavity could be formed when another electron mirror is placed slightly behind the back focal plane of the grating mirror. If a sample were placed inside this cavity, repeated weak interactions with the reflected/diffracted electron beam can be used to image the sample while keeping beam-induced sample damage arbitrarily low.

The approach outlined here will be used to characterize diffraction from the patterned mirror surface. Demonstration of electron diffraction from a patterned surface in a FESEM will represent a significant advancement toward the demonstration of a QEM system.

3:20pm **NS+BI-ThA4 Nanoscale Chemical Imaging by Photo-induced Force Microscopy*, Ryan Murdick,*** Molecular Vista

Nanoscale Chemical Imaging with Photo-induced Force Microscopy

Sung Park

Molecular Vista, Inc.

Infrared Photo-induced Force Microscopy (IR PiFM) is based on an atomic force microscopy (AFM) platform that is coupled to a widely tunable mid-IR laser. PiFM measures the dipole induced at or near the surface of a sample by an excitation light source by detecting the dipole-dipole force that exists between the induced dipole in the sample and the mirror image dipole in the metallic AFM tip. This interaction is strongly affected by the optical absorption spectrum of the sample, thereby providing a significant spectral contrast mechanism which can be used to differentiate between chemical species. Due to its AFM heritage, PiFM acquires both the topography and spectral images concurrently and naturally provides information on the relationship between local chemistry and topology. Due to the steep dipole-dipole force dependence on the tip-sample gap distance, PiFM spectral images have spatial resolution approaching the topographic resolution of AFM, demonstrating sub 10 nm spatial resolution on a variety of samples. PiFM spectral images surpass spectral images that are generated via other techniques such as scanning transmission X-ray microscopy (based on synchrotron source), micro confocal Raman microscopy, and electron microscopes, both in spatial resolution and chemical specificity. The breadth of the capabilities of PiFM will be highlighted by presenting data on various organic, inorganic, and low dimensional materials. By enabling imaging at the nm-scale with chemical specificity, PiFM provides a powerful new analytical method for deepening our understanding of nanomaterials and facilitating technological applications of such materials.

Bio: Sung Park is the CEO of Molecular Vista, which he co-founded with Prof. Kumar Wickramasinghe (UC Irvine, formerly of IBM) in 2011 to provide research and industrial tools for rapid and nanoscale imaging with chemical identification. Sung has 25 years of experience of industrial R&D, engineering, marketing and sales, and operations. Sung co-founded Park Scientific Instruments (PSI), which was one of the first commercial companies to develop and sell scanning tunneling microscopes (STM) and atomic force microscopes (AFM); PSI was acquired by Thermo Instruments in 1997, by which point PSI had sold upwards of 1,000 instruments to customers worldwide. Prior to founding Park Scientific Instruments, Sung worked as a post-doc at IBM Watson Research Center. Sung earned a Ph.D. in Applied Physics from Stanford University and BA in Physics from Pomona College.

4:00pm **NS+BI-ThA6 Strong Coupling of Localized Surface Plasmon Resonances to Light-Harvesting Complexes from Plants and Bacteria*, A. Tsargorodska, M. Cartron, C. Vasilev,*** University of Sheffield, UK*; G. Kodali,* University of Pennsylvania*; J. Baumberg,* University of Cambridge, UK*; PL. Dutton,* University of Pennsylvania*; CN. Hunter,* University of Sheffield, UK*; P. Torma,* University of Aalto*; **Graham Leggett**,* University of Sheffield, UK

Plants and bacteria harvest solar energy with extraordinary efficiency. In chloroplasts, the quantum efficiency, defined as the fraction of captured photons that goes on to cause charge separation, is estimated to be ca. 90%. The mechanisms by which such extraordinary efficiencies are realised have been the subject of intense interest. We have explored the potential offered by plasmonic techniques for the investigation of biological light harvesting complexes. Macroscopically extended arrays of gold nanostructures are fabricated by interferometric exposure of an alkylthiolate SAM on gold, enabling the fabrication of macroscopically extended arrays of gold nanostructures in a rapid, simple process. After annealing, these structures yield strong localized surface plasmon resonances (LSPRs). In contrast to the behaviour observed for most proteins, the LSPRs are split when light-harvesting membrane proteins from purple bacteria and plants are attached to the gold nanostructures, yielding pronounced changes in their extinction spectra. The splitting is large, and is different for mutant proteins containing different pigment molecules, indicating that it is sensitive to the electronic structures of the membrane proteins. The splitting is attributed to strong coupling between the LSPRs and excitons in the light-harvesting complexes. The splitting is suggestive of an asymmetric Fano-type resonance, and the plasmon-exciton coupling has been modelled with coupled harmonic oscillators. The model yields good fits to the experimental spectra. It indicates that in light harvesting complexes 1 and 2 (LH1/2) from purple bacteria, coupling to the

carotenoid *S2* state dominates, with a strength of ~ 0.2 eV. However, in a carotenoid-free mutant of LH1 the LSPR couples with a strength of ~ 0.1 eV to the bacteriochlorophyll Q_x transition, which has a smaller transition dipole moment than do the carotenoids. The coupling varies with the square root of the surface coverage of the protein, consistent with strong coupling theory. Strong coupling was also observed for self-assembling polypeptide maquettes that contain only chlorins. However, it was not observed for monolayers of bacteriochlorophyll, indicating that strong plasmon-exciton coupling is sensitive to the specific presentation of the pigment molecules.

4:20pm **NS+BI-ThA7 Microfluidic Device For Aptamer-Based Cancer Cell Capture And Genetic Mutation Detection**, ***Sarah Reinholt***, *H.G. Craighead,* Cornell University

Genetic mutations in cancer cells are not only fundamental to the disease, but can also have tremendous impact on the efficacy of treatment. Identification of specific key mutations in a timely and cost-effective way would allow clinicians to better prescribe the most effective treatment options. Here, we present a novel microfluidic device as a platform for specifically capturing cancer cells and isolating their genomic DNA (gDNA) for specific amplification and sequence analysis. To filter out rare cancer cells from a complex mixture containing a diversity of cells, nucleic acid aptamers that specifically bind to cancer cells are immobilized within a microchannel containing micropillars to increase capture efficiency. The captured cells are then lysed and the gDNA is isolated via physical entanglement within a secondary micropillar array. This type of isolation allows the gDNA to be retained within the channel, and enables multiple consecutive rounds of isothermal amplification in which different individual genes are amplified separately. The amplified gene samples undergo sequencing, and the resulting sequence information is compared against the known wildtype gene to identify any mutations. Cervical and ovarian cancer cells have been initially tested for mutations in the *TP53* gene using this technology. This approach offers a way to monitor multiple genetic mutations in the same small population of cells, which is beneficial given the wide diversity in cancer cells, and requires very few cells to be extracted from the patient sample. With this capability for genetic monitoring, precision medicine should be more accessible for the treatment of cancer.

4:40pm **NS+BI-ThA8 Molecular Processes in an Electrochemical Clozapine Sensor**, ***Thomas Winkler***, University of Maryland, College Park; *S.L. Brady,* East Carolina University; *E. Kim,* University of Maryland, College Park; *H. Ben-Yoav,* Ben-Gurion University of the Negev, Israel; *D.L. Kelly,* University of Maryland, Baltimore; *G.F. Payne, R. Ghodssi,* University of Maryland, College Park

Selectivity presents a crucial challenge in electrochemical sensing. One example is schizophrenia treatment monitoring of the redox-active antipsychotic clozapine (CLZ). To accurately assess efficacy, differentiation from its metabolite *N*-desmethylclozapine (NDMC) – similar in structure and redox potential – is critical. Here, we leverage biomaterials integration to study, and effect changes in, diffusion and electron transfer kinetics of these compounds. A key finding in our present work is differing dynamics between CLZ and NDMC once we interface the electrodes with chitosan-based biomaterial films. These additional dimensions of redox information can thus enable selective sensing of largely analogous small molecules.

Our study utilizes gold working electrodes either bare, coated with chitosan, or with our previously demonstrated redox cycling system (RCS). In the RCS, electrodeposited chitosan serves as a matrix to immobilize electroactive catechol near the electrode *via* electrografting. Small redox species diffuse through the film for oxidation at the electrode; the nearby catechol enables subsequent reduction of the analyte, establishing a signal-amplifying redox cycle. We execute cyclic voltammetry at 1m—10V/s sweep rates with CLZ, NDMC, or the model redox couple 1,1′-ferrocenedimethanol (FC).

With bare gold, both CLZ and NDMC exhibit similar (R^2=0.99) drastic increases in peak separation even at 0.5V/s, indicating slow electron transfer kinetics, in contrast to FC (Nernstian up to 3V/s). With both chitosan and the RCS we find that similarity broken. For diffusion, the coefficients D reveal two regimes in chitosan: dominance of bulk solution below 10mV/s (values match those from bare gold and theory), and diffusion inside the film becoming limiting at higher scan rates. This is reflected in *D* decreasing by 1.9× for FC, 17× for CLZ, and 31× for NDMC. The sharp difference between FC and the other larger two suggests a size-restriction phenomenon. The consistently 2× lower *D* for NDMC over the similarly sized CLZ points to possible electrostatic effects. With the RCS, signal amplification translates into apparent *D* increases – 9× over bare for FC, 5× for CLZ, and 3× for NMDC. Only at high scan rates does *D* decrease toward the chitosan-only value as true diffusion asserts dominance.

In conclusion, our results demonstrate the intricate interplay between biomaterials, biomolecules, and electrochemistry. They reveal intriguing distinguishing characteristics of CLZ from both the largely analogous NDMC as well as the model FC. This opens up avenues of utilizing diffusion and kinetics information to enhance selectivity in electrochemical sensing.

5:00pm **NS+BI-ThA9 Quantitative Quartz Crystal Microbalance Measurements across Transients Produced by Switching Fluid Properties**, *V. Mugnaini,* ***Dmitri Petrovykh***, International Iberian Nanotechnology Laboratory, Portugal

We systematically investigated Quartz Crystal Microbalance with Dissipation (QCM-D) measurements in aqueous solutions of model strong electrolytes that are commonly used in experiments with biological surfaces. In particular, we examined the quantitative behavior of both frequency and dissipation responses in transitions between two different aqueous solutions.

The abrupt changes in the QCM-D responses upon such transitions are sometimes referred to as “jumps” associated with switching the bulk properties of the fluid flowing through the QCM-D cell. Switching between fluids of different compositions may be important in a variety of QCM-D measurements for biointerfaces, e.g., when switching between a baseline/rinsing solution and a measurement solution, or switching between optimal buffers used for probe immobilization and for biorecognition steps [1-3]. In specialized quantitative biointerface measurements, such as measuring stabilities of DNA hybrids [2-3], switching among different solutions multiple times actually provides the basis for the measurement.

In typical QCM-D measurements, the baseline is reset after a “bulk jump”, so the data are typically only quantified between any transients, but not across them, i.e., quantification is carried out for a constant fluid composition, but not between different fluids. By considering the underlying viscoelastic formalism [4-5], we demonstrate in a series of systematic measurements for solutions of strong electrolytes that the QCM-D responses upon switching between different solutions can be quantitatively predicted and exhibit interesting scaling behavior. Classical theory of the viscosity of electrolyte solutions provides additional insight into correlations between the results measured for different salts.

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5:20pm **NS+BI-ThA10 ToF-SIMS/XPS Characterization of Frozen-Hydrated Hydrogels**, ***Michael Taylor***, *M.R. Alexander,* The University of Nottingham, UK; *M. Zelzer,* National Physical Laboratory, UK

Over the last decade the beneficial properties of hydrogels as artificial cell culture supports have been extensively investigated[1]. Certain synthetic hydrogels have been proposed to be similar in composition and structure to the native extracellular matrix of the stem cell niche, their *in vivo* cell habitat, which is a powerful component in controlling stem cell fate[2]. The stem cell differentiation pathway taken is influenced by a number of factors. When culturing cells within or upon hydrogels this choice can be strongly dependent on the underlying 3D hydrogel chemistry which strongly influences hydrogel-cell interactions[3]. The interrelationship between hydrogel chemistry and that of biomolecules in controlling cellular response ideally requires analysis methods to characterise the chemistry without labels and often in 3D. Time-of-flight secondary ion mass spectrometry (ToF SIMS) has the potential to be utilised for through thickness characterisation of hydrogels. The frozen-hydrated sample format is well suited to minimise changes associated with dehydration or the chemical complexity of ‘fixation’, a challenging aspect in vacuum analysis conditions[4]. Frost formation can occur in the ambient atmosphere preventing ready depth profiling of the frozen hydrogels[5]. We develop a simple method to remove this frost by blowing with gas prior to entry into

the instrument which is shown to produce remarkably good profiles on a poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel film where a model protein, lysozyme, is incorporated to demonstrate how biomolecule distribution within hydrogels can be determined. A comparison of lysozyme incorporation is made between the situation where the protein is present in the polymer dip coating solution and lysozyme is a component of the incubation medium. It is shown that protonated water clusters $H(H_2O)_n^+$ where n=5-11 that are indicative of ice are detected through the entire thickness of the pHEMA and the lysozyme distribution through the pHEMA hydrogel films can be determined using the intensity of characteristic fragment secondary ions. We also expand the developed methodology to X-Ray Photoelectron Spectroscopy (XPS) for through thickness analysis of the similar pHEMA / lysozyme hydrogels, and show that lysozyme distribution can be quantitatively mapped in hydrogels.

5:40pm **NS+BI-ThA11 GCIB-SIMS for Studying Bacterial Surfaces***, **John Stephen Fletcher**, P. Wehrli,* University of Gothenburg, Sweden*; A. Farewell,* University of Gothenburg, Seweden*; T.B. Angerer, J. Gottfries,* University of Gothenburg, Sweden

For many years ToF-SIMS has shown the promise of delivering new information of direct relevance to biological research. However, inadequacies in the ability to generate intact molecular ion species and then detect them with precise mass resolution and accuracy have held the technique back. Recent advances in ToF-SIMS, through the implementation of gas cluster ion beams (GCIBs) coupled to non-conventional MS systems, now permit the analysis of higher mass species from native, underivatised, biological specimen i.e. intact bacterial cells. Being able to characterise and understand changes in bacterial biochemistry as a result of environmental, biological or pharmacological stress is critical to address the global challenge of antibiotic resistance. For example, *E. coli* is able to rapidly adjust the biophysical properties of its membrane phospholipids to adapt to environmental challenges including starvation stress. Here, these membrane lipid modifications were investigated in glucose starved *E. coli* cultures and compared to a D*rel*AD*spo*T ($ppGpp^0$) mutant strain of *E. coli,* deficient in the stringent response, by means of time-of-flight secondary ion mass spectrometry (ToF-SIMS Cultures in stationary phase were found to exhibit a radically different lipid composition as compared to cultures in exponential growth phase. Wild-type *E. coli* reacted upon carbon starvation by lipid modifications including elongation, cyclopropanation and increased cardiolipin formation. Observations are consistent with variants of cardiolipins (CL), phosphatidylglycerols (PG), phosphatidylethanolamines (PE), phosphatidic acids (PA), and fatty acids. Notably, despite having a proteomic profile and a gene expression profile somewhat similar to the wild-type during growth, the $ppGpp^0$ mutant *E. coli* strain was found to exhibit modified phospholipids corresponding to unsaturated analogues of those found in the wild-type. We concluded that the $ppGpp^0$ mutant reacts upon starvation stress by elongation and desaturation of fatty acyl chains, implying that only the last step of the lipid modification, the cyclopropanation, is under stringent control. These observations suggest alternative stress response mechanisms and illustrate the role of the RelA and SpoT enzymes in the biosynthetic pathway underlying these lipid modifications.

Thin Film
Room 104E - Session TF+BI-ThA
Thin Films for Bio-related Applications

Moderator: Angel Yanguas-Gil, Argonne National Lab

2:20pm **TF+BI-ThA1 Self-healing Antifouling Fluorinated Monolayers and Polymer Brushes: One Fluorine Goes a Long Way!***, **Zhanhua Wang**, H. Zuilhof,* Wageningen University, Netherlands

Organic monolayers or polymer brushes, often in combination with surface structuring, are widely used to prevent nonspecific adsorption of polymeric or biological material on sensor and microfluidic surfaces. Here we show how robust, covalently attached alkyne– derived monolayers or ATRP-produced polymer brushes, with a varying numbers of fluorine atoms, on atomically flat Si(111), effectively repel a wide range of apolar polymers without the need for micro– or nanostructuring of the surface. We have studied the antifouling property of fluoro-hydro monolayers and of fluorine-containing polymer brushes towards a range of commonly used polymers/plastics with comparable molecular weight in non– aqueous solvent, and have investigated the effect of polymer molecular weight on the fouling behavior. In addition, we show how for fluorinated polymer brushes this property can be self-repaired upon damage. These studies relied on a range of characterization methods: wettability studies, ellipsometry, X– ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). We developed a novel surface morphology survey by AFM characterization that can accurately quantify the degree of fouling.

These findings and analysis offer significant potential for antifouling applications of ultrathin and covalently bound fluorine– containing coatings for a range of micro– and nanotechnological applications.

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2:40pm **TF+BI-ThA2 Sensitivity Enhancement in Grating Coupled Bloch Surface Wave Resonance by Azimuthal Control***, **Vijay Koju**, W.M. Robertson,* Middle Tennessee State University

Bloch surface waves (BSWs) are electromagnetic excitation modes that exist at the interface of truncated dielectric multilayer structures and a homogeneous medium. Although BSWs are intrinsically present at such interfaces, they cannot be directly excited by light incident from the homogeneous medium due to their non-radiative and evanescent nature. The use of a grating coupler or a prism mitigates this inability by providing an additional momentum to the free-space wave vector required to satisfy the phase matching condition with the BSW wave vector. Since Grating-coupled Bloch surface wave resonance (GCBSWR) bio-sensors do not require a bulky prism to couple light into BSWs, they are strong candidates for nanoscale bio-sensors. But GCBSWR bio-sensors, based on either wavelength or angular interrogation, are observed to be less sensitive compared to prism-coupled Bloch surface wave resonance (PCBSWR) bio-sensors. However, due to their inhomogeneous surface architecture, GCBSWR bio-sensors can be interrogated by rotating the grating platform azimuthally. Exploiting this ability, here we present a new method for improving sensing capability of GCBSWR bio-sensors. We demonstrate computationally, using a three-dimensional scattering matrix based rigorous coupled wave analysis method, that the proposed azimuthal angle interrogation technique highly enhances the sensitivity of GCBSWR bio-sensors. For our study we use a sixteen layered TiO_2-SiO_2 multilayer with SiO_2 gratings on the top sensing platform. We fix the wavelength and incident angle of the incoming light, and sweep over the azimuthal angle to simulate the sensitivity as a function of changing refractive index of the sensing layer. Furthermore, we show that contrary to conventional GCBSWR bio-sensors that only work for transverse electric mode, azimuthal angle based GCBSWR bio-sensors work for both transverse electric and transverse magnetic modes.

4:00pm **TF+BI-ThA6 Thin Film Technologies for Biomedical Devices-Current State of Art and Future Opportunities***, **Mallika Kamarajugadda**,* Medtronic plc **INVITED**

Thin film coatings are becoming ubiquitous in the medical device industry. Capabilities of medical devices and implants are greatly enhanced by thin films, which impart different properties such as adhesion, wear resistance, corrosion resistance, lubricity, radiopacity, electrical insulation, and bio response. Thin film deposition processes for medical devices are often challenging due to the complex substrate geometry of the components and the requirement for biocompatibility. In biomedical thin film coatings, the shape of a surface controls its interaction with biological components. Optimizing the interactions that occur at the surface of implanted biomaterials will be the key to further advances in this field. Furthermore, as treatment options shift towards non-invasive methods, and device size is reduced, researchers will need to work towards overcoming technological challenges to leverage thin film technology in medical devices. This talk will provide current examples of thin film coating applications in the medical device industry along with the future opportunties.

4:40pm **TF+BI-ThA8 Preparation and Characterization of Amino Coatings for Peptide Arrays***, **Gaurav Saini**, L. Howell, M. Greving, P. Walsh, D. Smith,* HealthTell Inc. **INVITED**

Amine-functionalized substrates are among the most commonly used materials in solid-phase peptide synthesis. Chemical stability and amine loading of the amino coating are two important properties that determine silane selection as a building layer in peptide synthesis. We synthesized three different amino coatings *i.e*., APTES (3-aminopropyltriethoxysilane), APDEMS (3-aminopropyldiethoxymethylsilane) and APDIPES (3-aminopropyldiisopropylethoxysilane), and determined their strengths and limitations as a building layer in peptide array synthesis. Here, amino coatings were synthesized via gas-phase deposition of the corresponding silanes on thermal oxide-terminated silicon substrates in a commercial

chemical vapor deposition system. A 16-mer peptide coating was then synthesized on the amino surfaces and the chemical stability of the surface to highly acidic side chain deprotection (SCD) treatments was determined. After SCD, the coating thicknesses decrease to different degrees on the surfaces: it is greatest for the APDIPES surface, lowest for the APTES surface and intermediate for the APDEMS surface, which indicates that peptide-functionalized APTES and APDIPES surfaces are chemically most and least stable to SCD treatment, respectively. The effect of amine loading on peptide density and purity was also determined for the three amino surfaces. Four different trimers were synthesized on the amino surfaces, and the density and purity of these trimers for the three surfaces was determined. A positive correlation between the amine loadings and peptide densities was observed; peptide density was highest for the APTES surface and lowest for the APDIPES surface. However, high amine loading is found to have a negative impact on peptide purity; peptide purity is highest for the APDIPES surface and lowest for APTES surface. Coated surfaces were characterized by spectroscopic ellipsometry, contact angle goniometry, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), spectrophotometry, and MALDI-MS.

5:20pm **TF+BI-ThA10 Electronic Characterization of SWCNT/Block Copolymer-based Nanofiber for Biosensor Application**, ***Amrit Sharma,*** Clark Atlanta University

The aim of this research is to fabricate an electrically conducting, smooth, continuous and sensitive nanofiber using polystyrene (PS), triblock copolymer (PS-b-PDMS-b-PS) and single-walled carbon nanotubes (SWCNTs) by electrospinning. The electronic nanofibers may be utilized for effective bio-sensing applications. The SWCNTs have been of great interest to researchers because of their exceptional electrical, mechanical, and thermal properties. The nanoscale diameter, high aspect ratio, and low density make them an ideal reinforcing candidate for novel nano composite material.

Electrically conducting nanofibers have been prepared by electrospinning a solution of PS, PS-b-PDMS-b-PS and functionalized SWCNTs in the ratio 5:1:0.05 using solvent DMF. The nanofibers formed had an average diameter of 5 µm and height 4 µm. These nanofibers were characterized by scanning electron microscopy (SEM), atomic force microscopy (AFM), optical microscopy and electrical characterization.

The electrical characterization of a single fiber shows an almost linear graph of current vs voltage using four-point probe (also known as Kelvin sensing) method. This linear graph exemplifies the conducting nature of the nanofiber. From the graph, a resistance, resistivity and conductivity of the single were measured. The study suggests that the SWCNT/block copolymer nanofibers have superior performance in the development of ultra-high sensitive sensor for the detection of single molecule relative to conventional materials due to significantly larger surface-to-volume ratio. Future work includes preparing nanofibers decorated with functional groups and binding with specific type of enzyme or protein to study their I-V behavior. This approach or method can be utilized for bio-sensing activities, especially for the detection of various antibodies and protein molecules.

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Faculty Advisor/ Mentor: Dr. Michael D. Williams, mdwms@cau.edu

Tribology Focus Topic
Room 101A - Session TR+BI+SE+TF-ThA
Materials Tribology

Moderator: Michael Chandross, Sandia National Laboratories

2:20pm **TR+BI+SE+TF-ThA1 Reaction Pathways and Tribofilm Formation Kinetics at a Solid-Solid Interface**, *H.L. Adams,* University of Wisconsin-Milwaukee*; A. Martini,* University of California Merced*;* ***Wilfred Tysoe,*** University of Wisconsin-Milwaukee **INVITED**

Perhaps the most difficult surface-science challenge is to monitor reaction pathways and kinetics at sliding solid-solid interfaces, in particular for opaque contacting materials [1]. Optical techniques can be used to interrogate the interface when one of the contacting surfaces is transparent, but they are often not sensitive to the first monolayer. Strategies for measuring reaction pathways and their kinetics for well-defined surfaces in ultrahigh vacuum (UHV) are described using the example of sliding-induced decomposition of adsorbed methyl thiolate species, formed by exposure to dimethyl disulfide, on copper. Surface science experiments show that methyl thiolates are stable up to ~425 K on copper, but decompose during rubbing; the effect of the external force is to lower the reaction activation barrier so that it proceeds at room temperature. The surface reaction products can be monitored immediately after sliding in UHV using surface spectroscopies (for example, Auger spectroscopy). However, the reaction kinetics can also be monitored in situ first, by measuring the gas-phase species evolved as a function of the number of times the surface is rubbed, where methane and ethane are detected and second, by measuring the change in friction force due to the evolution of the nature of the species present on the surface. This allows the elementary steps in the tribofilm formation pathway to be identified and their rates measured.

[1] Heather L. Adams, Michael T. Garvey, Uma Shantini Ramasamy, Zhijiang Ye, Ashlie Martini, and Wilfred T. Tysoe, Journal of Physical Chemistry C, **119**, 7115–7123 (2015)

3:00pm **TR+BI+SE+TF-ThA3 Nanotribology of Graphene Revisited: The Influence of Contact Size and Substrate Topography**, *A. Balkanci,* Bilkent University, Turkey*; Z. Ye, A. Martini,* University of California Merced*;* ***Mehmet Z. Baykara,*** Bilkent University, Turkey

Two-dimensional (2D) materials have been the focus of intense research in recent years thanks to their outstanding electronic and mechanical attributes. In particular, graphene exhibits exceptional potential as a solid lubricant appropriate for use in nano-/micro-scale mechanical systems. As such, a comprehensive evaluation of its frictional properties on such small length scales is of crucial concern. While pioneering studies toward this purpose have revealed strongly layer-dependent frictional behavior [1], the precise roles that contact size and substrate topography (important design parameters for mobile components in nano-/micro-scale devices) play in the lubricative nature of graphene have not been explored yet in detail.

In this contribution, we present a combined experimental and numerical study aimed at evaluating the influence of contact size and substrate topography on the nanotribological characteristics of graphene. In particular, atomic force microscopy (AFM) is employed under ambient conditions to measure friction forces on mechanically-exfoliated graphene as a function of applied load, number of graphene layers, and contact size. To study the influence of contact size on measured frictional properties, AFM probes with different tip apex sizes are obtained by thermal evaporation of gold and platinum onto the probes. In conjunction with the experiments, molecular dynamics (MD) simulations are performed that involve the calculation of friction forces experienced by model tip apexes of varying size on single- and few-layer graphene. Moreover, substrates with various RMS roughness and correlation length values are employed in the MD simulations to investigate the effect of substrate topography on frictional behavior. Results reveal that a subtle interplay between contact size and substrate topography determines the layer-dependent frictional behavior of graphene, providing a new perspective to the nanotribology of this remarkable material.

[1]: C. Lee *et al.*, Science **328**, 76 (2010)

3:20pm **TR+BI+SE+TF-ThA4 Iron-Doped Diamond-Like Carbon Coatings (Fe-DLCs): Synthesis, Characterization, and Tribology--Seminal Results**, ***Parag Gupta,*** Northwestern University/Argonne National Lab*.; M.E. Graham,* Northwestern University

Iron-doped diamond-like carbon coatings (Fe-DLCs) of ≈ 0.1 to 35 at.% Fe content have been synthesized, characterized, and tribologically tested.

Coatings were deposited on Si(111), 52100 steel ball, and H-13 steel flat substrates using a closed-field unbalanced magnetron sputter deposition process with unmodified and modified graphite target states, the latter with press-fit cast gray iron slugs. Process parameters of target modification, target power, acetylene flowrate, and substrate bias were varied and used in establishing a process-conditioning window to create predictable coatings.

Mechanical characterization was done to determine deposition rate, thickness, internal stress, and hardness. Cross-sectional characterization was done to determine coating uniformity, to understand coating adhesion and morphology, and to confirm interlayer presence and morphology (if deposited). Surface characterization was done to determine surface roughness and mechanical anisotropy. Chemical characterization was done to determine elemental concentration and chemical anisotropy. Finally, structural characterization was done to determine carbon bond order.

Using a ball-on-flat reciprocating tribometer, highly-doped Fe-DLCs were studied at either room temperature or ≈ 100 °C and with either coating / coating or steel / coating contact. Electrical contact resistance between interfaces was measured *in situ*. A contact pressure of ≈ 1 GPa was employed alongside an average sliding speed of 1.0 cm / s, except when non-monotonic sequential speed stepping was prescribed. The boundary-lubricated sliding tests were conducted in the presence of poly-alpha-olefin SAE grade 30 synthetic base stock oil (PAO10) with and without molybdenum dithiocarbamate (MoDTC) and zinc dialkyldithiophosphate (ZDDP) additives, both at 0.5 wt.%. Coatings were also tested in unlubricated conditions.

Friction responses were determined, and wear assessments were conducted. Tribofilm and debris analyses were done. The results were compared to those from DLC, CrN + DLC, Si-DLC, and W-DLC coatings obtained from Oerlikon Balzers. Results indicate that Fe-DLC samples containing between 12 and 35 at.% Fe exhibit negligible wear in the presence of PAO10 with MoDTC and ZDDP, affirming the influence of iron in catalyzing protective tribofilms. Additionally, wear on such samples in both lubricated and unlubricated conditions is far lower than that observed for other coatings, indicating that these Fe-DLCs are robust in any conditions.

4:00pm **TR+BI+SE+TF-ThA6 Tribo-Rheometry of Soft Matter***, J. Kim,* ***Alison Dunn,*** University of Illinois at Urbana-Champaign **INVITED**

Hydrogel surfaces are biomimics for sensing and mobility systems in the body such as the eyes and large joints due to their compliance, controllable chemistry, permeability, and integrated aqueous component. Recent studies have shown that polymer concentration gradients in the top microns of crosslinked hydrogel surfaces result in a less dense surface region. In addition, the lubrication of hydrogel interfaces is driven by the effective mesh size, a parameter which follows from the local density. Given the similarity of a dilute crosslinked hydrogel surface with a dilute polymer solution, we probe the surface of a polyacrylamide hydrogel using stepped-velocity tribo-rheometry over 5 decades of sliding speed, with an annular aluminum countersurface. Three distinct lubricating regimes emerge based on a) hysteretic torque response depending upon increasing or decreasing sliding speeds, and b) characteristic torque overshoot following velocity step changes. This evidence supports the analogy of a rheology-like lubrication response. We postulate that the mechanisms of hydrogel-against-hard material lubrication are due to distinct complex fluid behavior characterized by weakly or strongly time-dependent response. Tribo-rheometry is particularly suited to uncover the lubrication mechanisms of complex interfaces such as are formed with hydrated hydrogel surfaces and biological surfaces.

4:40pm **TR+BI+SE+TF-ThA8 Friction Coefficient Lowering in High-hardness Boron Nitride Films Under Ultra-high Vacuum***, Masao Noma,* Shinko Seiki Co., Ltd, Japan*; K. Eriguchi,* Kyoto University, Japan*; M. Yamashita,* Hyogo Prefectural Institute of Technology, Japan*; S. Hasegawa,* Osaka University, Japan

Solid lubricant material with low friction coefficient is of technological interest for its usage under harsh environments such as ultra-high vacuum. At present, MoSi -containing films [1] are the most widely employed for space applications because of their low friction coefficients (0.02–0.05) in vacuum [2]. However, the mechanical hardness and the oxidation resistance temperature are 10–20 GPa [3] and 360 °C [4], respectively, inapplicable to a long term operation in space. Boron nitride (BN) films have been considered an alternative material because of their superior high hardness and oxidation resistance temperature, 45 GPa and 1200 °C, respectively [5]. We have proposed a novel reactive plasma-assisted coating technique (RePAC) for forming 1-μm-thick high-hardness BN films (~50 GPa) [6][7]. In this study, we present "friction coefficient lowering" phenomena in the high-hardness BN films under ultra-high vacuum (~10^{-6} Pa), which is in sharp contrast to "friction coefficient increase" usually observed for other hard coating materials. The time-dependent high-vacuum friction measurement revealed that the friction coefficient decrease from 0.1 to 0.03 was found for the substrate bias voltage from −90 to −180 V in the RePAC. In this (incident ion energy) region, the cubic BN phase was formed in the turbostratic BN background, leading to the high-hardness of ~50 GPa at atmosphere [7]. Moreover, the obtained low friction coefficient was confirmed to be stable (<0.05) for long time exposures to the vacuum (~96 hrs). The friction coefficients of the present BN films are comparable to widely reported values of MoS_2 films. The BN film prepared by the RePAC is one of promising hard coating materials for harsh environment (e.g., space) applications.

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5:00pm **TR+BI+SE+TF-ThA9 Nanoscale Friction Properties of Water Intercalated Graphene on Mica and its Isotope Effects***, Hyunsoo Lee,* Institute for Basic Science (IBS) & Korea Advanced Institute of Science and Technology (KAIST)*; J.-H. Ko,* KAIST, Republic of Korea*; J.S. Choi,* Electronics and Telecommunications Research Institute, Republic of Korea*; J.H. Hwang,* IBS & KAIST, Republic of Korea*; Y.-H. Kim,* KAIST, Republic of Korea*; M.B. Salmeron,* Lawrence Berkeley National Laboratory (LBNL)*; J.Y. Park,* IBS & KAIST, Republic of Korea

We demonstrate that the frictional behavior of hydrophobic graphene on hydrophilic mica is affected by water intercalation after exposure to humid air using atomic force microscopy. The single- and multi-layer graphene were formed by mechanical exfoliation on freshly cleaved muscovite mica. The adsorption of the ice-like water layer between graphene and mica led to friction enhancement, as compared with a pristine graphene/mica sample, which is presumably due to additional frictional energy dissipation at the solid–liquid interface. Moreover, friction on the graphene increased as the number of stacking water layers increased. The magnitude of friction increase was, on the other hand, reduced as following increase of the number of covering graphene layer above intercalated water layer, and then the friction is eventually not distinguished from the multi-layer graphene stack excluded water adsorption. Using the first-principle density functional theory calculations we explain this unexpected behavior by the increased spectral range of vibration modes of graphene caused by water, particularly the low frequency flexural modes, and by the better overlap of the graphene vibration modes with the mica phonons, which favors a more efficient dissipation of the frictional energy. Additionally, we found that the intercalation of deuterium oxide (D_2O) leads to the lower friction, compared to H_2O intercalated graphene on mica. We attribute this isotope effect with to the low vibrational frequency of D_2O adsorbate, which results in the low rate of frictional energy dissipation at the interface.

5:20pm **TR+BI+SE+TF-ThA10 The Remarkable Friction Behavior of Copper at Cryogenic Temperatures***, Andrew Kustas,* Sandia National Laboratories*; J. Curry,* Lehigh University*; T. Babuska, M. Chandross, P. Lu, T.A. Furnish, N. Argibay,* Sandia National Laboratories

It is commonly accepted that unlubricated, self-mated pure metal contacts over the course of sliding invariably cold-weld and gall, leading to undesirably high friction and wear. Recent work with nanostructured pure metals has shown that in fact it is possible to obtain low friction ($\mu < 0.5$) with pure bare metals such as pure Cu and Au at room temperature. Here we discuss those findings, and more recent work that shows the impact of temperature, stress and microstructure evolution on friction of self-mated pure metals. Variable temperature friction experiments were used to show the existence of a temperature-dependent transition for Cu from high ($\mu > 1$) to low ($\mu = 0.25$) friction, achieved by sufficiently reducing temperature and promoting the development of nanocrystalline surface films that are unachievable at room temperature at the relatively high applied stresses imposed. In-situ electrical contact resistance (ECR) measurements were used to indirectly measure the evolution of the microstructure (grain size) at the interface throughout the experiment. Microscopy was then used to

verify claims of nanocrystalline surface film formation at low temperatures. Lastly, an analytical model based exclusively on materials properties is presented that incorporates stress and temperature over time to predict grain size, connecting grain size to friction behavior, for pure FCC metals. While more work is needed to develop the proposed framework, a model that intrinsically connects grain size to friction behavior of metals based exclusively on materials properties is transformational to alloy design, and raises a number of compelling and highly fundamental questions for further research.

5:40pm **TR+BI+SE+TF-ThA11 Understanding Friction in MoS2, Part 1: Stress, Time and Temperature***, **Tomas Babuska**,* Sandia National Laboratories*; J. Curry,* Lehigh University*; M. Chandross, M.T. Dugger,* Sandia National Laboratories*; B. Krick,* Lehigh University*; N. Argibay,* Sandia National Laboratories

In the 90 years since the first patent was issued for molybdenum disulfide (MoS_2) as a friction and wear reducing additive, great strides have been made in understanding its remarkable lubricity. However, much remains to be understood about the mechanisms of friction at the molecular scale. Firstly, we present results of investigations into the origins of the well-known non-Amontonian behavior of MoS_2. We show that the apparent return to Amontonian behavior previously reported with steel is in fact associated with an elasto-plastic transition of the contact, and that the stress-dependent friction predictable varies as a function of substrate composition and microstructure (hardness). Time-dependent friction evolution (i.e. run-in behavior) was also found be strongly a function of substrate material composition and stress; these results imply a potentially useful connection between stress and microstructure evolution in both film and substrate that is discussed. We also report on investigations into the temperature-dependent friction and wear behavior of pure MoS_2. In the range -150 to 250°C, we report dramatic deviations from previous literature, as well as the existence of transitions between thermal and athermal behavior as a function of temperature. Evidence of deviations from classical Arrhenius behavior is presented, and the implications of these findings discussed in the context of thermally-activated friction models at the molecular scale. Finally, we end with a discussion of how these findings collectively advance our ability to develop a practical predictive friction model for MoS_2 that includes temperature, stress, substrate effects, defect density and commensurability as their foundation.

6:00pm **TR+BI+SE+TF-ThA12 Understanding Friction in MoS2, Part 2: Water, Oxidation and Run- in***, **John Curry**,* Lehigh University*; M. Chandross, T. Babuska,* Sandia National Laboratories*; N.C. Strandwitz, H. Luftman,* Lehigh University*; M.T. Dugger, N. Argibay,* Sandia National Laboratories*; B. Krick,* Lehigh University

Effects of water vapor and oxidation resistance for amorphous (sputtered) and highly ordered (N_2 sprayed) MoS_2 were investigated with a high-sensitivity, low energy ion scattering (HS-LEIS) spectrometer, molecular dynamics simulations and accompanying tribological testing in each environment of interest. Recent studies have shown that N_2 sprayed MoS_2 coatings possess a preferential surface parallel basal plane texture as deposited due to the kinetic energy imparted during spraying, effectively shearing MoS_2 particles onto the surface. As such, the highly ordered structure of the sprayed coatings both at the surface and throughout the bulk of the film are hypothesized to act as a diffusion barrier to environmental contaminants. Coatings were exposed to molecular oxygen at 250°C and atomic oxygen at 20°C for 30 minutes each and subsequently depth profiled in the HS-LEIS. Results show that N_2 sprayed coatings were successful in limiting the depth of oxidation for both types of exposure. The main contributor, however, to increased initial friction post exposure was the type of coating (amorphous vs highly oriented). Tribological experiments in dry and humid nitrogen showed the initial friction response to be unaffected for sprayed samples while greatly affected for sputtered. Spiral orbit tribological testing was utilized in dry and humid nitrogen environments to further assess the effect of prolonged sliding on purely amorphous MoS_2 with and without formation of a transfer film. It is hypothesized that water does not poison friction behavior of established films of highly oriented MoS_2, but it does poison the ability to form long range order and sintering of crystallites.

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