Industrial Physics Forum
Room 101B - Session IPF+AS+BI+NS-MoM

Biofabrication: From Tissue to Organ

Moderators: Jason Bardi, American Institute of Physics, Jim Hollenhorst, Agilent Technologies

8:20am **IPF+AS+BI+NS-MoM-1 Strategic Thinking on the Architecture and Design of Scaffolds for Regenerative Medicine*, Buddy D. Ratner,*** University of Washington, Seattle **INVITED**

Scaffolds for use in medicine and biology might be traced back to the 1940's when parachute cloth was first used for vascular prostheses. However, in the mid-1980's scaffolds took off as an essential tool in tissue engineering. This talk will explore some of the basic biology of porosities, roughness and textures on cell responses in vitro and tissue responses in vivo. University of Washington studies will be presented demonstrating enhanced healing and regeneration with precision control of pore structures for in vivo applications. The use of surface techniques and tools will be addressed for decorating the surfaces of scaffolds with biological molecules. Finally, the potential of secondary ion mass spectrometry (SIMS) for analyzing and imaging pore structure will be addressed.

9:00am **IPF+AS+BI+NS-MoM-3 Sequential Bottom-up Assembly of Synthetic Cells*, Joachim Spatz,*** Max Planck Institute for Medical Research, Germany **INVITED**

The evolution of cellular compartments for spatially and temporally controlled assembly of biological processes became an essential step in developing life. Synthetic approaches towards cellular-like compartments are still lacking well-controlled functionalities as would be needed for more complex synthetic cells. In part, this is due to the mechanical and chemical instabilities of the lipid-based protocells and a lack of technical means for their well-controlled manipulation. We developed droplet supported lipid bilayer vesicles by microfluidics to generate mechanically and chemically stable and, therefore, manipulable cell-like compartments with a well-defined chemical and biophysical microenvironment. The enhanced stability enabled the sequential loading of such compartments with biomolecules by pico-injection microfluidics without compromising their functionality as synthetic cells. We demonstrate a successful sequential bottom-up assembly of a compartment with lipids, transmembrane proteins (integrin, F_oF_1-ATP synthase) and cytoskeleton proteins which would not assemble in a fully functional way by mixing and including them in one pot at once

9:40am **IPF+AS+BI+NS-MoM-5 Activation of Inkjet Printed Cells Enhances Microvasculature Formation in Host Tissues*, Thomas Boland,*** *B Oropeza, L Solis,* University of Texas at El Paso*; M Yanez,* University of South Carolina **INVITED**

Bioprinting refers to the co-deposition of cells alongside scaffolding materials to build two- and three-dimensional constructs for tissue engineering applications. The technology faces several limitations that present interesting engineering opportunities. The nature and scope of the problems will be discussed in the context of the fabrication of microvasculature. The current tissue-engineering paradigm is that successfully engineered thick tissues must include vasculature. Studies of membrane properties of thermal inkjet printed cells by evaluating showed normal electrophysiology, but short-term membrane disruptions, which allow small molecular weight molecules to enter. Cell viability was high and apoptotic behavior was not upregulated. Alginate (1%) and gelatin type B (2.5%) constructs or scaffolds were prepared by bioprinting of a crosslinker with endothelial and endothelial / β cells. Control scaffolds were manually pipetted with the same cells and without any cells.Upon implantation the bioprinted endothelial cell constructs showed a nearly ten-fold increase in blood vessels was observed (p= 0.009), a dose response was observed but the β cells seemed to inhibit vessel formation. The explanted implants show large complete vascular features on the H&E and CD31 stains; Immunohistochemistry showed the tissue were regenerated with the human cells that made up a large part of the vasculature. Further insights into how the inkjet printing process activated endothelial cells will be presented. Understanding these processes will improve bioprinting and may eventually lead to creating fully vascularized large soft tissues, which have not been successfully grown thus far.

10:40am **IPF+AS+BI+NS-MoM-8 Challenges in Organ-specific Vascular Engineering and Tissue Assembly*, Ying Zheng,*** University of Washington **INVITED**

Engineered tissues have emerged as promising new approaches to repair damaged tissues as well as to provide useful platforms for drug testing and disease modeling. Outstanding challenges remain in 1) the lack of well-defined and mature cell sources to facilitate translational outcomes and 2) the lack of control over vascular structure and perfusion efficiency in engineered 3D tissue constructs, preventing large-scale tissue fabrication, and leading to insufficient perfusion after implantation in vivo. In this talk, I will present recent progress in my lab in engineering microvasculature from human pluripotent stem cell derived endothelial cells, and their anastomosis *in vitro* and infarcted heart *in vivo*. The eventual goal of this drive is to use the single cell source to derive organ-specific vascular cells and tissue for regeneration. Next I will discuss our work in understanding the human microvascular endothelial cell heterogeneity from four major organs, heart, lung, liver and kidney and describe their distinct structure and function. I will show an example of using human kidney-specific microvascular cells to model kidney specific injury. Finally I will discuss challenges and future perspectives towards engineering human organ-specific tissue models.

11:20am **IPF+AS+BI+NS-MoM-10 Bioprinting for Translational Applications: *The Quest for Whole Organ Fabrication*, *James J. Yoo,*** Wake Forest School of Medicine **INVITED**

Tissue engineering and regenerative medicine has emerged as an innovative scientific field that focuses on developing new approaches to repairing cells, tissues and organs. Over the years, various engineering strategies have been developed to build functional tissues and organs for clinical applications. However, challenges still exist in developing complex tissue systems. In recent years, 3D bioprinting has emerged as an innovative tool that enables rapid construction of complex 3D tissue structures with precision and reproducibility. This developing field promises to revolutionize the field of medicine addressing the dire need for tissues and organs suitable for surgical reconstruction. In this session novel and versatile approaches to building tissue structures using 3D printing technology will be discussed. Clinical perspectives unique to 3D printed structures will also be discussed.

Biomaterial Interfaces Division
Room 101B - Session BI+AS+IPF+MN-MoA

Advanced Imaging and Structure Determination of Biomaterials Research

Moderators: Dan Graham, University of Washington, Axel Rosenhahn, Ruhr-University Bochum

1:20pm **BI+AS+IPF+MN-MoA-1 NMR Relaxometry as a Medical Diagnostic***, **Michael J. Cima,*** Massachusetts Institute of Technology **INVITED**

This talk will describe the diagnostic capabilities of magnetic resonance imaging (MRI) when brought to the patient bedside. Rather than imaging, NMR can be used for important chemical/physiologic diagnostic endpoints. Two will be discussed here; quantifying fluid overload and measurement of hypoxia within tumors. Assessment of intra- and extra-vascular volume is integral in managing patients with heart, liver, and kidney disease as volume status is closely linked to mortality. Commonly used determinants of volume status, such as physical exam and ultrasonography, lack sensitivity and specificity and require expertise in clinical practice. This talk reports on nuclear magnetic resonance (NMR) methods to a portable and clinically useful device. A clinical study with hemodialysis patients and age-matched healthy controls was performed at MGH. The T2 relaxation times of study participants' legs were quantified at multiple time points with both a 1.5T clinical MRI scanner and a custom 0.27T single-voxel MR sensor. The results showed that first sign of fluid overload is an increase in the relative fraction of extracellular fluid in the muscle. The relaxation time of the extracellular fluid in the muscle eventually increases after more fluid is accumulated. Importantly, these MR findings occur before signs of edema are detectable on physical exam. Solid tumors are often hypoxic and characterized by an extreme lack of oxygen. Tumor hypoxia imparts significant negative outcomes for patients but is highly variable within cancer types and patient populations. Many of these poor clinical outcomes can be tied to hypoxic-induced radiotherapy resistance. Resistance to radiotherapy in hypoxic regions can be overcome by increasing the dose delivered but exposure limitations of healthy tissue and organs must be considered. The lack of a viable quantitative clinical oxygen measurement method prevents safe dose escalation in these patient populations. Here we report on a silicone-based quantitative oxygen sensor. The MRI contrast of this material depends on dissolved oxygen. Thus, the material functions as a first of its kind solid-state contrast agent. The sensor leverages the existing MRI hardware, which is part of the current clinical work flow, to map tumor oxygen content. This information can then be integrated into the dose planning process clinicians currently conduct to selectively and safely boost dose to low oxygen tumor subvolumes. This sensor is approved by the institutional review board at Dana Farber Cancer Center for a clinical trial in patients locally advanced cervical cancer.

2:00pm **BI+AS+IPF+MN-MoA-3 Direct Observation of Cell Signaling Proteins Interacting with a Model Cell Membrane by Sum Frequency Generation Vibrational Spectroscopy***, T Golbek,* Oregon State University*; T Weidner,* Aarhus University, Denmark*; C Johnson, **Joe Baio,*** Oregon State University

Proteins that contain C2 domains are involved in a variety of biological processes including encoding of sound, cell signaling, and cell membrane repair. Of particular importance is the interface activity of the C-terminal C2F domain of otoferlin due to the pathological mutations known to significantly disrupt the protein's lipid membrane interface binding activity, resulting in hearing loss. Therefore, there is a critical need to define the geometry and positions of functionally important sites and structures at the otoferlin-lipid membrane interface. Here we describe the first *in situ* probe of the protein structure of otoferlin's C2F domain interacting with a cell membrane surface. To identify this protein's structure at the lipid interface we applied sum frequency generation (SFG) vibrational spectroscopy and coupled it with simulated SFG spectra to observe and quantify the otoferlin C2F domain interacting with model lipid membranes. A model cell membrane was built with equal amounts of phosphoserine (PS) and phosphocholine (PC). SFG studies that examined the ordering of the lipids that make up the model membrane, demonstrate that lipid fusion occurs after docking of the otoferlin C2F domain via the observation of a 62% increase in amplitude from the SFG signal near 2075 cm^{-1} assigned to specific groups within the model membrane. This increase is related to lipid ordering caused by the docking interaction of the otoferlin C2F domain. SFG spectra taken from the amide I region contain peaks near 1621 cm^{-1} and 1672 cm^{-1} related to the C2F domains beta-sandwich secondary structure, thus, indicating that the domain binds in a specific orientation. By mapping the simulated SFG spectra to the experimentally collect SFG spectra, we found the C2F domain of otoferlin orients 32° normal to the lipid surface. This information allows us to map what portion of the domain directly interacts with the lipid membrane. Furthermore, we show first experimental view of any C2 domain of otoferlin docked at the membrane interface, thereby, validating SFG as a method to probe C2 domain-membrane interfaces.

2:20pm **BI+AS+IPF+MN-MoA-4 Vibrational Sum-frequency Scattering Spectroscopy for the Characterization of Protein Fiber Structures and their Surface Interactions in Biological Environments***, **Patrik K. Johansson**, D Castner,* University of Washington

Biological processes are typically regulated by interactions at the interface of 3D structures, such as the membrane of cells or protein fiber surfaces. Collagen (the most common protein in mammals) forms large fibers that are responsible for the structural integrity of tissues. The structure, organization and interactions of these fibers are furthermore important for the survival, communication, migration, and proliferation of cells.

Investigating protein fiber interactions is challenging, particularly under biological conditions where the fibers exist in a 3D aqueous environment. Many techniques cannot interrogate interfaces buried in the bulk of a solvent and therefore require 2D surface models, while others need extensive purification and sample preparation. These approaches may not capture all key characteristics of the fiber surface structure and interactions in the real sample. However, vibrational sum-frequency scattering (SFS) spectroscopy, with inherent contrast for local molecular ordering, can be utilized towards these important goals.

As a first demonstration, we have applied SFS to protein fibers in aqueous environments, self-assembled from collagen type I. We detected signals from the amide I band and the N-H stretching vibrations, both of which are related to the specific protein backbone structure. Signals from the C-H stretching and bending vibrations were also identified, which are more associated with the side-chains in the fibers. The angular scattering patterns for the backbone (amide I) and side-chain (C-H stretches and bends) signals are different, making the spectra dependent on the angle of detection. While the backbone signals are dominant in the phase-matched direction, the side-chain signals remain high also at large scattering angles. Distinctions in the organizational symmetry and the relative fiber surface contribution to the overall signal are hypothesized as reasons for this observation.

Finally, we are investigating the impact of changes to the environment (e.g. ionic strength, pH, surfactants) on the shape of spectra and scattering patterns for the detected SFS signals. This could yield new insights to the structure and dynamics of collagen fibers in biological settings. The relevance of such investigations is enhanced by the fact that detection of vibrations from the surrounding molecules is a direct observation of their interactions with the collagen fiber surface, which thus can be correlated with the fiber structure. The relative orientations for the detected groups can also be obtained via vibrational SFS polarization analysis, for a deeper understanding of biomolecular interactions in biological processes.

2:40pm **BI+AS+IPF+MN-MoA-5 How Proteins Grow Calcium Carbonates – The Mechanism of Vaterite Bioprecipitation Studied at the Molecular Level by Sum Frequency Generation Spectroscopy***, H Lu,* Max Planck Institute for Polymer Research, Germany*; S Roeters,* Aarhus University, Denmark*; H Lutz, M Hood, A Schäfer,* Max Planck Institute for Polymer Research, Germany*; R Muñoz-Espí,* Universidad de Valencia, Spain*; M Bonn,* Max Planck Institute for Polymer Research, Germany*; **Tobias Weidner**,* Aarhus University, Denmark

Proteins can act as Nature's engineers at interfaces and manipulate hard tissue growths. Specialized peptides can bind and release specific mineral facets and grow the intricate mineral morphologies found in diatom cell walls, mollusk nacre, but also human teeth and bone. Taking clues from Nature we aim at understanding the mineralization processes at the molecular level and to develop design rules for biogenic nanophase materials. Mineral proteins control the biogenesis of $CaCO_3$ by selectively triggering the growth of calcite, aragonite or vaterite phases. The templating of $CaCO_3$ by proteins must occur predominantly at the protein/$CaCO_3$ interface. Surprisingly, molecular-level insights into the interface during active mineralization have been lacking. Here, we investigate the role of peptide folding and structural flexibility on the mineralization of $CaCO_3$. We discuss the mineral activity of amphiphilic

peptides based on glutamic acid and leucine with *ß*-sheet and *α*-helical secondary structures. While both sequences lead to vaterite structures, the *ß*sheets yield free-standing vaterite nanosheet with superior stability and purity. Surface-specific spectroscopy studies and molecular dynamics simulations reveal that the interaction of calcium ions with the peptide monolayer restructures both the peptide backbone and side chains. This restructuring enables effective templating of vaterite by mimicry of the vaterite (001) crystal plane. The approach is universally applicable to mineral peptide engineering. We will discuss how analogous peptide designs can be used to steer the growth not only of calcium carbonates but also calcium oxalates.

3:00pm **BI+AS+IPF+MN-MoA-6 ToF-SIMS Imaging of Chemical Modifications in Topographically Challenging Materials***, **Michael Taylor**, D Graham, L Gamble,* University of Washington

Three-dimensional (3D) porous materials are applied in a variety of areas within materials science[1]. Pores in catalysts provide a high surface reaction area, pores in biofilters facilitate fluid movement for biomolecule capture, and pores in tissue engineered constructs allow for cellular ingress and vascularization. These applications require surface modifications to add specific functionality to their surfaces. The successful functionality of these materials is related to the ability of these modifications to reach all surfaces of the pores. However, it is challenging to characterize these complicated materials and verify the presence and distribution of these surface modifications. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) is a powerful label-free surface analysis tool that can be used to image the molecular composition of cells, tissues and polymers. Porous 3D materials however, are non-ideal for ToF-SIMS analysis as the technique is highly surface-sensitive, topography on the order of microns can inhibit the ability to produce secondary ions related to surface modifications. To solve this problem we have developed a methodology for filling voids in porous materials to produce a surface where ToF-SIMS imaging may be performed. A embedding process for porous materials with poly(vinyl alcohol)(PVA) is detailed followed by freezing and cryo-sectioning to expose the modified scaffold interior. Here, we demonstrate the versatility of this method by high spatial resolution 3D imaging of a number of surface modifications in PCL poly(caprolactone) scaffolds[2]. Characterisation of fluorocarbon (FC) films deposited using octofluoropropane (C3F8) plasma enhanced chemical vapor deposition (PECVD) will be demonstrated, showing that increased treatment times deposits uniform coatings while shorter treatment results in a gradient distribution of FC throughout the PCL scaffold. Additionally we show data on imaging immobilized/adsorbed proteins within PCL scaffolds. Using this methodology we demonstrate that high spatial resolution label-free 3D imaging of chemical modifications in materials with complex geometries is now possible with ToF-SIMS.

Refs:

(1) Yang, X.-Y.; Chen, L.-H.; Li, Y.; Rooke, J. C.; Sanchez, C.; Su, B.-L. Hierarchically Porous Materials: Synthesis Strategies and Structure Design. *Chem. Soc. Rev.***2017**, *46* (2), 481–558 DOI: 10.1039/C6CS00829A.

(2) Taylor, M. J.; Aitchison, H.; Hawker, M. J.; Mann, M. N.; Fisher, E. R.; Graham, D. J.; Gamble, L. J. Time of Flight Secondary Ion Mass Spectrometry—A Method to Evaluate Plasma-Modified Three-Dimensional Scaffold Chemistry. *Biointerphases***2018**, *13* (3), 03B415 DOI: 10.1116/1.5023005.

3:40pm **BI+AS+IPF+MN-MoA-8 Imaging Plant and Plant Growth-Promoting Bacteria Interactions Using Time-of-Flight Secondary Ion Mass Spectrometry***, **Xiao-Ying Yu**, R Komorek, Z Zhu, C Jansson,* Pacific Northwest National Laboratory

We present the first imaging and spectra results of plant root interactions with plant growth-promoting bacteria (PGPB) using time-of-flight secondary ion mass spectrometry (ToF-SIMS), showing the successful application of delayed image extraction to study plant biology. Compared to MALDI (Matrix Assisted Laser Desorption Ionization), an imaging mass spectrometry technique widely used in plant studies,[1] SIMS is less destructive and provides submicrometer spatial mapping of molecular species of importance in metabolic processes. *Brachypodium distachyon* (Brachypodium), a genomics model for bioenergy and native grasses, is used due to its small diploid genome, close phylogenetic links to other grass species, relative ease of genetic transformation, short life cycle, small stature, and simple growth requirements.[2] Plant growth-promoting bacteria (PGPB) such as *Pseudomonas* and *Arthrobacter* were introduced to Brachypodium roots prior to analysis, and their potential effect on root extrusion was studied using ToF-SIMS imaging. Specifically, delayed image extraction was used in data acquisition. This approach was chosen to obtain high mass and high spatial resolutions.[3] Excellent SIMS imaging gives topographical description of the root surface with and without PGPB interactions. Distinctive characteristic peaks are observed, indicating compositional changes with and without PGPB introduction to the root surface beside visible surface morphological variations. Our initial results demonstrate that ToF-SIMS is a promising imaging mass spectrometry tool to study plant biology and root-microbe interactions and provide molecular-level insight at the biointerface with high spatial resolution.

References:

[1] D Sturtevant *et al.*, Three-dimensional visualization of membrane phospholipid distributions in Arabidopsis thaliana seeds: A spatial perspective of molecular heterogeneity, Biochimica et Biophysica Acta (2017),**1862**(2)*,* 268-81.

[2] T Girin *et al.*, Brachypodium: a promising hub between model species and cereals, J. Experimental Botany (2014),**65**(19)*,* 5683-96*.*

[3] QP Vanbellingen *et al.*, Time-of-flight secondary ion mass spectrometry imaging of biological samples with delayed extraction for high mass and high spatial resolutions, Rapid Comm. Mass Spectrom. (2015), **29** (13), 1187-95.

4:00pm **BI+AS+IPF+MN-MoA-9 Imaging of Cells and Tissues with Helium Ion Microscopy***, J Notte, D Wei, **Chuong Huynh**,* Carl Zeiss Microscopy, LLC

Both optical and electron microscopy are well established techniques in the life sciences with established protocols for imaging and sample preparation. However the newly developed helium ion microscope has some unique advantages, and is gaining a reputation for providing insightful, easy to interpret images over a wide range of biological samples and bio-materials. This presentation serves as both an introduction to this novel technique and a review of recent results.

Because helium ions do not suffer appreciably from diffraction effects, they can be focused to a sub-nanometer probe, providing nanometer scale image resolution with a depth of focus that is well suited to complex surfaces and structures. As helium ions interact with the sample, they provide an abundance of secondary electrons that convey surface-specific and topographical information. Distinctly different from the conventional (gallium) focused ion beams, helium ions do not significantly damage the sample from the sputtering process. And importantly, helium ion microscopy is not affected by charging artifacts when imaging insulating materials, even glass slides, so there is no need for metal over-coating which would otherwise obscure finer details.

Example images will include a pancreatic cell membrane showing the pores and cilia present on their natural surfaces. Other examples will show the complex structure of the principal cell and intercalated cells of the collecting duct of a rat kidney. Other imaging results from diverse fields include stony corals, collagen networks, bone minerals, stereocilia, otoconia, actin filaments, and cryptococcus neoformans. False colorized images of the multi-ciliated epithelial trachea of an adult mouse and T4-phages will also be presented. Finally, new results will be shown from the SIMS spectrometer which provides elemental and isotopic information, and can be the basis for true colorizaton.

In this talk, an emphasis will be placed on the physics principles that enable these imaging results. The selected examples serve to demonstrate the breadth of results that can be attained with this relatively new technique.

4:20pm **BI+AS+IPF+MN-MoA-10 Quantitative Analysis of Electrolytes in Microliter-size Blood Drops Congealed via HemaDrop™ using Ion Beam Analysis and SIMNRA***, **Harshini Thinakaran**, S Narayan, J Day, N Herbots, F Ark, B Wilkens, M Mangus, R Culbertson,* Arizona State University

Accurate analysis of microliter blood samples can improve medical testing and forensics. Most critically ill patients suffer from hospital-acquired anemia due to the large volume currently required for blood diagnostic tests: 7 mL per vial.

Prior attempts by Theranos to analyze microliter-sized blood droplets in liquid form exhibit systematic errors greater than 10%, higher than the acceptable medical threshold.

This research investigates the accuracy of Ion Beam Analysis (IBA) performed on microliter-sized blood droplets congealed into Homogenous Thin Solid Films (HTSFs) using HemaDrop™, a new patent-pending technique using hyper-hydrophilic coatings to condense fluids into a uniform solid state with a smooth surface.

Prior to IBA analysis, the solidification of blood droplets into HTSF's is observed with optical microscopy and compared to conventional Dried Blood Spots (DBS). DBS exhibit phase separation between platelets and

serum, with non-uniform, rough surfaces. Conversely, blood droplets solidified on HemaDrop™-coated surfaces are uniform and smooth, with little phase separation.

Next, quantitative compositional analysis using IBA is performed on µL blood drops solidified on HemaDrop™ coatings and is compared to results on DBS. HTSFs congealed on HemaDrop™-coated surfaces yield well-defined 2 MeV RBS spectra where individual species and electrolytes (C, N, O, Na, K, Ca, Cl, Fe) can be identified, while none can be distinguished on DBS.

The damage curve method [1] extracts elemental composition while accounting for possible IBA damage. Several consecutive spectra are taken on the sample, and RBS yields are interpolated to their original concentrations.

IBA simulations with the software SIMNRA enable comparison between RBS data and simulations, resulting in elemental composition accurate within 1%. Blood electrolyte compositions via SIMNRA are obtained on successive IBA spectra taken on different areas of the thin solid films and on different HTSFs congealed from the same blood. Relative error analysis between different HTSF samples establishes whether reproducibility within 10% can be achieved.

HemaDrop™ reliably creates stable, uniform, thin solid films to measure blood composition from µL-volume drops based on comparative IBA results and optical observations. Measurements of elemental composition of HTSF of blood samples are accurate and reproducible. HemaDrop allows for analysis in vacuo from µL of blood, greatly expanding the range of techniques that can be applied to identify elements and molecules (e.g., antibiotics, proteins).

[1] *Int & US Patent Pending, 2016, 2017

Industrial Physics Forum
Room 101B - Session IPF+AS+BI+MN-TuM

Advanced Imaging and Structure Determination of Biomaterials

Moderators: David G. Castner, University of Washington, Michael Grunze, Max Planck Institute for Medical Research

8:00am **IPF+AS+BI+MN-TuM-1 Chemical Imaging as a Tool to asses Molecular and Morphologic Content in Natural Tissues and Fabricated Models**, ***Rohit Bhargava***, *T Comi, M Gryka,* University of Illinois at Urbana-Champaign **INVITED**

Chemical imaging, in which molecular content is obtained using spectroscopy and images are formed using microscopy, is an emerging area to characterize cells and tissues. We present here a chemical imaging approach based on mid-infrared spectroscopic imaging that combines the spatial specificity of optical microscopy with the molecular selectivity of vibrational absorption spectroscopy. IR spectroscopic imaging is particularly attractive for the analysis of cells and tissue in that it permits a rapid and simultaneous fingerprinting of inherent biologic content, extraneous materials and metabolic state without the use of labeled probes. Recorded data are related to the structural and functional state of the biological material using computation. We describe the computational strategy and statistical considerations underlying decision-making for this modality. A combination of theory, novel instrumentation and signal processing forms an integrated approach to biochemical analyses. First, we describe attempts to automate histopathology without dyes or human input. Results indicate that a rapid assessment of tissue is possible. Applied to engineered 3D tissue models for breast tumors, we show that the imaging technology is useful in rapidly assessing culture quality and that the model systems can act to inform researchers about the involvement of different cell types in cancer progression. Finally, we integrate imaging observations with those from conventional biological experiments to provide a complete view of cancer progression in these systems.

8:40am **IPF+AS+BI+MN-TuM-3 Fluorescence Dynamics and Nonlinear Optical Imaging Methods for Biomedical Applications**, ***Alba Alfonso Garcia***, *L Marcu,* University of California at Davis **INVITED**

Generation of quality bioengineered tissue constructs, a main cornerstone for regenerative medicine, require new tools to monitor their maturation processes. Optical imaging, and in particular fluorescence dynamics and nonlinear optical techniques, provides the means for non-destructive, longitudinal, and quantitative evaluation. Using fiber optics and catheterized imaging systems these strategies are implemented with flexible geometries that allow investigations be performed outside of the realm of the microscope and the microscope slide, but instead *in situ*, on bioreactors, culturing wells and chambers, or even *in vivo*. Fluorescence dynamics and nonlinear optical imaging are especially well suited as they rely on intrinsic properties of the biomaterials to generate contrast. Tissue autofluorescence allows spectroscopic evaluation of tissue components, and the analysis of its temporal dynamics leads to functional analysis of tissue status. Additionally, nonlinear light-matter interactions probe vibrational and electronic energy levels that provide enhanced biochemical specificity of tissue constituents. All these approaches are compatible with label-free strategies, avoiding the addition of labeling agents onto already complicated samples. In this presentation, I will overview applications of fluorescence dynamics and nonlinear optical imaging including fluorescence lifetime imaging, two-photon fluorescence or second harmonic generation in tissue engineering. In particular, I will discuss tracking approaches to visualize recellularization processes on bioengineered vascular constructs. I will also characterize tissue composition of carotid arteries along their length based on their autofluorescence lifetime signals, and how this correlate with the structural protein composition of the vessel wall as evaluated by gold-standard biochemical assays. Finally, we will see how these methods are also applied in different fields such as the generation of cartilage-based implants, and the real-time discrimination of healthy versus diseased tissues in the context of cancer diagnostics.

9:20am **IPF+AS+BI+MN-TuM-5 Single Molecule Imaging of Receptor Signalling**, ***Katharina Gaus***, University of New South Wales, Australia **INVITED**

Antigen recognition by the T cell receptor (TCR) is a hallmark of the adaptive immune system. When the TCR engages a peptide bound to the restricting major histocompatibility complex molecule (pMHC), it transmits a signal *via* the associated CD3 complex. How the extracellular antigen recognition event leads to intracellular phosphorylation remains unclear.

We develop single-molecule localization microscopy (SMLM) apporaches and novel analysis to determine how spatial organization regulates signal initiation and propagation. For example, we used SMLM data to map the organization of TCR-CD3 complexes into nanoscale clusters and to distinguish between triggered and non-triggered receptor copies. We found that only TCR-CD3 complexes in dense clusters were phosphorylated and associated with downstream signaling proteins, demonstrating that the molecular density within clusters dictates signal initiation. This lead us to propose a model in which antigen recognition is first translated into receptor clustering and then the density of receptor nanoclusters is translated into signaling. This model may explain how T cells can respond to both the affinity and dose of pMHC molecules with a common signal transduction mechanism (Pageon et al. PNAS 2016). We also developed novel FRET sensors to monitor the rate of receptor clustering (Ma et al. Nat Commun 2017) and a sensor that reports membrane charges (Ma et al. Nat Biotech 2017) to understand how biophysical properties of the plasma membrane contribute to TCR signaling.

11:00am **IPF+AS+BI+MN-TuM-10 Developing a Google-earth View of Tumour Metabolism through Multiscale Molecular Imaging**, *J Bunch,* ***Rory T. Steven,*** National Physical Laboratory, UK **INVITED**

Mass spectrometry (MS) is one of the most powerful techniques for chemical analysis and when combined with an imaging modality allows molecular chemistry to be visualised in 2D and 3D, from the nano- to the macroscale, in ambient conditions and in real-time. There are numerous techniques each having different modes of operation including label-free and labelled analyses.

Cancer Research UK has identified that building an understanding of the inter- and intra- heterogeneity of tumours and their evolution over time and in response to therapy will require greater insight into the underlying biology, using *in vivo* and *in vitro* models and integrating biomarkers into both early- and late-phase trials. In 2017 the Grand Challenge programme was launched. Our collaborative action involves NPL, Imperial College London, The Beatson Institute, ICR, Barts Cancer Institute, The Francis Crick Institute, The University of Cambridge and AstraZeneca. Together we will develop a validated pipeline for multi-scale imaging of tumours collected from GEMMs and patients.

By pursuing a multiscale (organ to organelle) and multi-omics approach with a range of mass spectrometry imaging (MSI) techniques (MALDI, DESI, SIMS and ICP MS), we aim to deepen our understanding of the interplay of genes, proteins, metabolites and the role of the immune system in cancer development and growth.

This presentation will review early results and a discussion of the challenges associated with such a large, multi-technique, multi-site, mass spectrometry project.

11:40am **IPF+AS+BI+MN-TuM-12 X-ray Diffraction and Coherent Imaging with Nano-focused Radiation: A Multi-scale Approach from Biomolecular Assembly to Cell, Tissue and Organ**, ***Jan-David Nicolas***, *T Salditt,* University of Göttingen, Germany **INVITED**

X-rays deeply penetrate matter and thus provide information about the functional (interior) architecture of complex samples, from biological tissues and cells to novel composite materials. However, this potential of hard x-rays in view of penetration power, high spatial resolution, quantitative contrast, and compatibility with environmental conditions has to date not been fully developed, mainly due to significant challenges in x-ray optics. With the advent of highly brilliant radiation, coherent focusing, and lensless diffractive imaging this situation has changed. We show how nano-focused hard x-rays can be used for scanning as well as for full field holographic x-ray imaging of biological samples [1]. The central challenge of inverting the coherent diffraction pattern will be discussed and different reconstruction algorithms will be presented, from holographic techniques [2] to ptychography [3,4]. Next, we will present new approaches to treat the massive diffraction data recorded in scanning nano-diffraction experiments of cells and tissues [5].

By scanning the sample through the focused x-ray beam and recording full diffraction patterns in each scan point, structural parameters can be mapped throughout the cell or histological section [6], offering a 'diffraction contrast' by which one can localize also unstained biomolecular assemblies in cells and tissues, and at the same time investigate their structure. As an example, we address the sarcomeric organization in heart

muscle cells (cardiomyocytes) [7,8], and show how the sarcomere organization evolves and differs between different cell types and maturation states. As a multi-scale approach, we then discuss sarcomeric structure in heart tissue sections, and then finally present phase contrast tomography reconstructions of an entire mouse heart.

[1] Bartels et al., Phys. Rev. Lett. (2015), 114, 048103

[2] Krenkel et al., Acta Crystallogr. A (2017), 73, 282-292

[3] Giewekemeyer et al., PNAS (2010), 107, 529-534

[4] Wilke et al., Optics Express (2012), 20, 19232-19254

[5] Nicolas et al., J. Synchrotron Rad. (2017), 24, 1163-1172

[6] Carboni & Nicolas et al., Biomed. Opt. Express (2017), 8, 4331–4347

[7] Bernhardt et al., New J. Phys. (2017), 19, 013012

[8] Nicolas et al., J. Appl. Crystallogr. (2017), 50, 612–620

Biomaterial Interfaces Division
Room 101B - Session BI+AS+IPF+NS-TuA

IoT Session: Biofabrication, Bioanalytics, Biosensors and Diagnostics

Moderators: Graham Leggett, University of Sheffield, UK, Tobias Weidner, Aarhus University, Denmark

2:20pm **BI+AS+IPF+NS-TuA-1 Functionalization of Silica Materials *via* Click Reaction of Surface Silanol Groups with Vinyl Sulfones**, ***Fang Cheng***, *H Wang, W He, B Sun, J Qu,* Dalian University of Technology, China

Silica-based materials are widely used in the fields of catalysis, chromatography, biomaterials, biosensing and drug delivery due to their earth abundance and low cost. Success of these applications mostly relies on the functionalization of silica surfaces, among which covalent binding of organic molecules is preferred. Common strategies for the covalent functionalization of silica materials involve either silane treatments or Si-H reactions. Each has its share of limitations, with the former suffering from self-polymerization and multilayer modifications, and the latter being sensitive to moisture and oxygen. Herein, we proposed the 'click' reaction of silanol groups with vinyl sulfones, which enables a new and simple strategy for functionalization of silica materials. For the first time, the 'click' concept was extended to silanol groups that are abundant on the surface of silica materials, using compounds bearing vinyl sulfone groups. By simply immersing silica materials in vinyl sulfone solutions at 60℃, functionalization could be achieved in hours in the presence of catalysts. The chemical stability of vinyl sulfones and mild reaction conditions make this strategy advantageous than silane treatments and Si-H reactions. We demonstrated that silica materials with sizes ranging from microscale to macroscale could all be functionalized. Using compounds bearing multiple vinyl sulfone groups, silica materials can be further functionalized with varies of biomolecules due to the versatile reactivity of vinyl sulfone group towards thiol, amino and alcohols. Furthermore, the stability of resulting Si-O-C bond can be tuned by the properties of the vinyl sulfone compounds (e.g., hydrophobicity and surface density) as well as the environmental factors (e.g., solvents, pH and temperature). Increase in the hydrophobicity and functionalization density of the vinyl sulfone compounds could increase the stability of Si-O-C bonds. Contrast to the high stability in organic solvents, degradation of Si-O-C bond can be realized in aqueous solutions, which can be accelerated by addition of acid or base. This is rarely observed with bonds produced based of silane treatments and Si-H reactions. It could broaden the biomedical applications of functionalized silica, for example, to provide tailored release of drugs or proteins from silica surface.

2:40pm **BI+AS+IPF+NS-TuA-2 Organosilica pH Nanosensors Applied to Realtime Metabolite Monitoring**, ***Kye Robinson,*** Monash University, Australia*; K Thurecht,* University of Queensland, Australia*; S Corrie,* Monash University, Australia

Continuous monitoring of biomarkers in biological environments is a key challenge for the development of biosensors capable of providing real-time feedback[1]. These sensors promise to aid in the treatment of diseases with a highly dynamic nature however current technologies remain scarce[1]. Nanoparticle based "optodes" have emerged as sensitive and tuneable biosensors, using chromo/ionophores to generate analyte-specific changes in fluorescence spectra in a dynamic and reversible manner. Currently this type of sensor suffers from limitations including leaching of reagents from the nanoparticles over time, combined with poor colloidal stability and resistance to fouling in biological fluids.

An organosilica core-shell pH sensitive nanoparticle containing a mixture of covalently incorporated pH-sensitive (shell) and pH-insensitive (core) fluorescent dyes has been developed. This platform demonstrates good long term stability (80 days), fast response time (<100 ms) and resistance to fouling in biological conditions[2]. This presentation will describe the modification of these pH sensing particles towards the production of a lactate responsive particle for sensing through coupling with lactate dehydrogenase. Here we will present our latest results focussed on enzyme encapsulation in addition to modulation of shell parameters including thickness and degree of crosslinking in order to tune response kinetics for application in biological tissues.

[1] Corrie, S. R. et al., Analyst, **2015**, 140, 4350-4364

[2] Robinson, K. J. et al., ACS Sensors, **2018**

3:00pm **BI+AS+IPF+NS-TuA-3 Impact of Different Receptor Binding Modes on Surface Morphology and Electrochemical Properties of PNA-based Sensing Platforms**, ***Johannes Daniel Bartl,*** Walter Schottky Institut (WSI) and Physics Department, Technische Universität München, Germany*; P Scarbolo,* Dipartimento Politecnico di Ingegneria e Architettura (DPIA), Università degli Studi di Udine, Italy*; S Gremmo, G Rziga, M Stutzmann,* Walter Schottky Institut (WSI) and Physics Department, Technische Universität München, Germany*; M Tornow,* Molecular Electronics Group and Department of Electrical and Computer Engineering, Technische Universität München, Germany*; L Selmi,* Dipartimento di Ingegneria "Enzo Ferrari" (DIEF), Università di Modena e Reggio Emilia, Italy*; A Cattani-Scholz,* Walter Schottky Institut (WSI) and Physics Department, Technische Universität München, Germany

Silicon-based field-effect devices have been widely studied for label-free DNA detection in recent years. These devices rely on the detection of changes in the electrical surface potential during the DNA recognition event and thus require a reliable and selective immobilization of charged biomolecules on the device surface [1]. The preparation of self-assembled monolayers of phosphonic acids (SAMPs) on metal oxide surfaces is an efficient approach to generate well-defined organic interfaces with a high density of receptor binding sites close to the sensing surface [2,3]. In this work, we report the functionalization and characterization of silicon/silicon nitride surfaces with different types of peptide nucleic acid (PNA), a synthetic analogue to DNA [4].

Differently modified PNA molecules are covalently immobilized on the underlying SAMPs either in a multidentate or monodentate fashion to investigate the effect of different binding modes on receptor density and morphology important for PNA-DNA hybridization. Multidentate immobilization of the bioreceptors *via* C_6-SH attachment groups at the *γ*-points along the PNA backbone provides a rigid, lying configuration on the device surface (PNA 1), whereas a monodentate immobilization by Cys-capped PNA molecules (PNA 2) results in more flexible and more accessible receptor binding sites. Our results indicate that the presented functionalization scheme can be successfully applied to produce morphologically and electrochemically different PNA bioreceptor binding sites on silicon/silicon nitride surfaces. Consequently, a well-chosen modification of the PNA backbone is a valid approach to influence the sensing properties of surface-immobilized PNA bioreceptors, which might provide an additional parameter to further tune and tailor the sensing capabilities of PNA-based biosensing devices.

[1] Ingebrandt S. and Offenhausser A., *Phys. Status Solidi A* **203** (2006), 3399–3411.

[2] Chaki N. K. and Vijayamohanan K., *Biosens. & Bioelectron*. **17** (2002), 112.

[3] Stutzmann M., Garrido J. A., Eickhoff M. and Brandt M. S., *Phys. Status Solidi A* **203** (2006), 3424–3437.

[4] Nielsen P. E. and Egholm M. (ed.), Peptide Nucleic Acids, Horizon Scientific Press (1999).

3:20pm **BI+AS+IPF+NS-TuA-4 Biosensor for Detection of Gasotransmitter from Living Cells Employing Silver Nanorods Array**, ***Shashank Gahlaut,*** *C Sharan, J Singh,* Indian Institute of Technology Delhi, India

The detection of endogenous gases including H_2S is of immense interest nowadays as it opens the way to predict some diseases as well as an early stage diagnosis. These three gasotransmitter (H_2S, NO and CO) gaseous molecules transfer the information and give the signal for mainly cardiovascular diseases Therefore, its detection has crucial importance in bio-medical science. Here, we demonstrate H_2S detection from living cells using silver nanorods arrays fabricated by glancing angle deposition method. Colorimetric and wettability properties of silver nanorods are being observed for the gaseous detection. We use the model organism *E.coli* to demonstrate the feasibility of the method for the determination of live and resistant strains of the bacteria. For the human cell, we have used Hela cell line for the same. For the simplicity and feasibility of the technique, Android based mobile app has been developed for the colorimetric detection. Data obtained in this study show the potency of the system to identify live/dead bacteria with or without antibiotic treatment and compared with the time-consuming standard plating method, it a simple and cost-effective method for the estimation of living and resistant microorganism. The performance of AgNRs as H_2S gas sensor is investigated by its sensing ability of 5 ppm of gas with an exposure time of only 30 s. It has potential application in the area of antimicrobial resistance and bio-medical healthcare.

4:20pm **BI+AS+IPF+NS-TuA-7 Conversion of Human Stem Cells into Insulin Producing Cells Through 2D Platforms for Enhanced in-vitro Insulin Production**, *S Vishwakarma, A Khan,* Central Laboratory for Stem Cell Research and Translational Medicine, Centre for Liver Research and Diagnostics, Deccan College of Medical Sciences, India*; **Marshal Dhayal,*** IIT (BHU), Varanasi, India

Transplantation of whole pancreas/cadaveric islets is the most commonly acceptable treatment option for uncontrolled diabetes. However, the wider clinical applicability of these approaches is limited due to unavailability of donors and continuous need for the administration of immunosuppressant. Here we report a new strategy for efficient in-vitro trans-differentiation of human-hepatic progenitor cells (hHPCs) into insulin producing cells (iPCs) on biologically compatible micro-chips of 2D platforms. The physiological function of transdifferentiated hHPCs confirmed the activation of intracellular Ca^{++} signaling and activation of pancreatic transcription factors (pTFs) which triggers the insulin exocytosis during hyperglycemic challenge. The iPCs on these micro-chips showed upregulated expression of master regulator Pdx-1, β-cell specific marker Nkx-6.1 and more importantly C-peptide similar to human pancreatic b-cells during hyperglycemic challenge. These platforms may provide long-term survival and function of iPCs which could be better technology for developing effective therapeutic options for the management of diabetic.

4:40pm **BI+AS+IPF+NS-TuA-8 Polyzwitterion-modified Nanoparticles for Selective Antibody Separation**, *F Cheng, C Zhu, **Wei He**, B Sun, J Qu,* Dalian University of Technology, China

Antibody separation is a key biopharmaceutical process, which requires high specificity and efficiency in isolating the biomacromolecule from a complex biological fluid. Development of the separation adsorbent benefits diagnostics and therapeutics, such as point-of-care testing, treatment of cancer and autoimmune disease. In the process of antibody separation, Protein A chromatography is a commonly employed adsorbent, which could obtain antibody in high purity from serum or ascites. In the process-scale purification and therapeutic plasma exchange, safety issues, e.g. leakage and instability of the immobilized Protein A, and cross-contamination during regeneration, are overwhelmed in biopharmaceutics. An alternative approach to Protein A chromatography is using synthetic ligand, molecular weight of which is commonly less than 200 Da. The main advantages of synthetic ligand are well-controlled chemical structure, low cost, ease in clean-in-place, and repeatable regeneration capability in harsh conditions. However, it is a challenge to adsorb antibody in a highly selective manner from a complex biological fluid, which consists a variety of proteins with a broad range of concentrations.

Herein, we report a facile method to develop a quick separation adsorbent, which adsorbs antibody from a complex biological fluid with a high specificity. Two types of zwitterionic polymer-modified magnetic nanoparticles (NPs) are fabricated by conjugating pSBMA onto PEI-precoated NPs via either one-step method (1S NPs) or two-step method (2S NPs). For both methods, divinyl sulfone is used as linker molecule. Although 1S NPs were capable of resisting both IgG and BSA, 2S NPs exhibited specificity toward IgG adsorption in complex biological fluids, e.g. mixture of serums and IgG. The moderate interactions (Kd ~1.2 μM) between IgG and 2S NPs are three orders of magnitude lower than IgG binding with Protein A (Kd 10nM) . Through complementary characterizations and analyses, we rationalize that the surface developed herein with IgG specificity contains two key components: polyzwitterions with short chain length and sulfone groups with high density.

5:00pm **BI+AS+IPF+NS-TuA-9 Orienting Proteins on Surfaces with Site-specific Bioorthgonal Ligations**, ***Riley Bednar**, R Mehl,* Department of Biochemistry and Biophysics, Oregon State University

The functionalization of material surfaces with proteins is of great importance to a number of technologies, from industrial processes to biomedical diagnostics. However, while it has been proposed that orientation may be important to the function of such biomaterials, efforts to study such roles are hampered by a lack of rapid, quantitative, and orientation-specific immobilization techniques which will reduce non-specific fouling, and allow substoichiometric attachment of proteins onto surfaces in an orientation-controlled manner. Here, Carbonic Anydrase II (HCA)—a 30 kDA, monomeric metalloenzyme which catalyzes the interconversion of carbon dioxide to bicarbonate—is immobilized onto strained *trans*-cyclooctene (sTCO)-functionalized magnetic resin in an orientation-specific manner via bioorthogonal ligation with a site-specifically installed tetrazine-containing amino acid (Tet2.0).

5:20pm **BI+AS+IPF+NS-TuA-10 High-throughput Study of the Role of Spatial Organization on the Activity of Surface-Bound Enzymes**, ***Nourin Alsharif**,* Boston University*; T Lawton, J Uzarski,* Natick Soldier Research, Development and Engineering Center*; K Brown,* Boston University

Many of the exceptional properties of natural materials (e.g. fracture toughness of bones, strength to weight ratio of bamboo) can be attributed to their structural hierarchy, which originates, in part, from the nanoscale organization of the enzymes that synthesize these materials. In order to best utilize such enzymes *ex vivo* to grow engineered biomaterials, the role of this multiscale organization must be understood. Here, we report a novel strategy for studying the activity of arrangements of enzymes within a multifunctional material in a high throughput manner. In particular, we use top-down patterning techniques in conjunction with small molecule self-assembly to designate enzyme-binding regions amidst a non-binding, hydrophobic background. Key to this experimental scheme is the parallel nature of both the fabrication and the characterization processes that enable the efficient study of many geometric parameters of the enzyme-binding features. These parameters include, (1) feature size, (2) density of enzyme within each feature, and (3) distance between features. This level of control can in principle allow us to separate effects of reaction kinetics and substrate diffusion. Two strategies have been explored for the immobilization of enzymes including click chemistry to non-natural amino acids and binding to poly-histidine affinity tags. Top-down lithography and enzyme assembly were verified using a variety of surface characterization techniques including atomic force microscopy, X-ray photoelectron spectroscopy, infrared spectroscopy, spectroscopic ellipsometry, and contact angle goniometry. Initially, this high throughput paradigm is used to develop a fluorimetric assay to quantify the activity of surface-bound enzymes as a function of their spatial organization. Together with the widespread utilization of high throughput techniques in synthetic biology, the ability to study spatial organization in a rapid fashion is expected to dramatically improve *ex vivo* applications of enzymes.

5:40pm **BI+AS+IPF+NS-TuA-11 Fabrication of Amino acid Contained Poly-lactic Acid Nanofibers by Electrospinning**, *C Li,* National Yang Ming University, Taiwan, Republic of China*; J Hsieh,* Ming Chi University of Technology, Taiwan, Republic of China*; **P.H. Lin,*** National Yang Ming University, Taiwan, Republic of China

Polylactic acid (PLA, $[C_3H_4O_2]_n$, CAS 26161-42-2) is a biodegradable and thermoplastic polymer. PLA is naturally produced and can be extracted from many plants such as sugarcane, cornstarch or cassava roots. Typical industrial production processes for PLA are direct condensation of lactic acid monomers (~100°C - 160°C) and ring-opening polymerization of lactide with metal catalysts. For applications in bulk forms, PLA can be produced by extrusion, casting, injection molding and spin coating or even 3d printing.

In cell and tissue engineering applications, amino acids are essential ingredients for cell-tissue culture, implants/replacements, drugs and treatment tests. There are twenty amino acids appearing in human genetic codes by triplet codons and usually categorized according to their polarity, acidity/basicity.

In this study, we fabricate nanofibers by electrospinning on a spin-coated PLA film. This specially designed combination of PLA films and nanofibers is meant to have enduring interfacial adhesion between the two for biomedical applications such as implants. Both PLA nanofibers and films are mixed with selected amino acids. Five amino acids were chosen: tryptophan (Trp,), methionine (Met,), serine (Ser,), glutamate (Glu,) and arginine (Arg,). The selection is based on the different electrical polarity of each amino acid. The electrical polarity has profound effects on the solubility, pH acidity of amino acids in water and many other associated biochemical functions. These amino acids are representatives of certain biochemical features for potentially different influences in our applications for cell culture.

The electrospinning process is controlled by several parameters such as the voltage of power supply, feeding velocity of polymer solution through the syringe pump, electrical field strength and distance to the collection plate of nanofibers. Different combinations of these parameters are studied to determine an optimal control for fiber formation. Properties of and microstructures of deposited films and nanofibers are investigated as following: thickness and deposition rate by surface profilometer; microstructures by Fourier transform infrared spectrometer (FTIR); surface morphology by scanning electron microscope (SEM); optical properties by UV-Visible-IR spectrometer and wettability by the contact angle.

Industrial Physics Forum
Room 101B - Session IPF+AS+BI+NS-WeM

IoT Session: Bioanalytics, Biosensors and Diagnostics

Moderators: Anna Belu, Medtronic, Inc., Sally McArthur, Swinburne University of Technology, Australia

8:40am **IPF+AS+BI+NS-WeM-3 Harnessing Bacteria for Fabrication of Photoelectrodes and Pressure Sensors**, *Y Feng, K Marusak, Y Cao, E Ngaboyamahina, J Glass, L You,* ***Stefan Zauscher***, Duke University **INVITED**

Conventional methods for material fabrication often require harsh reaction conditions, have low energy efficiency, and can cause a negative impact on the environment and human health. In contrast, structured materials with well-defined physical and chemical properties emerge spontaneously in diverse biological systems. However, these natural processes are not readily programmable. By taking a synthetic-biology approach, we demonstrate a method for the fabrication of semiconducting, transition metal nanoparticles (NPs) with tunable bandgap and useful photoelectric properties, through bacterial precipitation. Surface analytic measurements revealed that our bacterially precipitated CdS NPs are agglomerates of quantum dots (QDs) in a carbon-rich matrix. We discovered that the precipitation conditions of the bacteria can be tuned to produce NPs with bandgaps that range from quantum-confined to bulk CdS. We determined the photoelectrochemical properties and energy band structure of thin films prepared from these NPs by electrochemical measurements. By taking advantage of the organic matrix, which is residual from the biosynthesis process, we fabricated a prototype photo-charged capacitor electrode by incorporating the bacterially precipitated CdS with a reduced graphene oxide sheet. Furthermore, we show the programmable, three-dimensional (3D) material fabrication using pattern-forming bacteria growing on top of permeable membranes as the structural scaffold. When the bacteria are equipped with an engineered protein that enables the assembly of gold (Au) nanoparticles into a hybrid organic-inorganic dome structure, the resulting hybrid structure functions as a pressure sensor that responds to touch. We furthermore show that the response dynamics are determined by the geometry of the structure, which is programmable by the membrane properties and the extent of circuit activation. By taking advantage of this property, we demonstrate signal sensing and processing using one or multiple bacterially assembled structures. Our work provides the first demonstration of using engineered cells to generate functional hybrid materials with programmable electronic properties and architectures for energy conversion, energy storage, and for signal sensing and transduction.

9:20am **IPF+AS+BI+NS-WeM-5 Surface Chemistry and Surface Analysis: Their Importance and Application in Industrial Genomics**, ***Fiona Black***, Illumina Inc. **INVITED**

Understanding the genome has the power to revolutionize health. However, building robust and scalable tools to interrogate single base variants with high robustness requires a system level approach to integrate surface patterning and activation, biosensing, and imaging. This talk will review how micro-patterning, bioanalytical controls, surface analytical techniques and measurement tools are applied in an industrial setting to develop and manufacture cutting edge systems for sequencing and genotyping applications

11:00am **IPF+AS+BI+NS-WeM-10 Design and Evaluation of Organosilica Nanosensors for Continuous Molecular Monitoring in Complex Biological Environments**, ***Simon Corrie***, Monash Univ., Melbourne AU, Australia **INVITED**

Continuous monitoring of biomarkers in biological environments is a key challenge for the development of biosensors capable of providing real-time feedback. Sensors capable of continuous pH monitoring have already found applications in detection of bacterial infections and have potential for aiding in treatment of dynamic diseases. Nanoparticle based "optodes" have emerged as sensitive and tuneable biosensors, using chromo/ionophores to generate analyte-specific changes in fluorescence spectra in a dynamic and reversible manner. Current key limitations of these materials include leaching of reagents from the nanoparticles over time, combined with poor colloidal stability in biological fluids.

Organosilica is a promising material for developing stable biosensors, allowing simple control over size, interfacial chemistry and porosity. This presentation will describe the development of a core-shell nanoparticle containing a mixture of covalently incorporated pH-sensitive (shell) and pH-insensitive (core) fluorescent dyes. Attachment of anti-fouling polymers is used reduce aggregation and biofouling in biological media. Fluorescence analysis of the nanoparticles reveals that the shell/core fluorescence ratio is highly sensitive to pH over a physiological range with the response time <1s. The sensitivity and dynamic range can be tuned by varying material properties of the shell (primarily thickness and porosity). We will present our latest results on the application of these nanosensors for continuous, real-time monitoring, including in bacterial cultures, subcutaneous mouse "tattoos," and in 3D hydrogel scaffolds.

11:40am **IPF+AS+BI+NS-WeM-12 Optoregulated Biointerfaces**, ***Aránzazu del Campo***, INM-Leibniz Institute for New Materials, Germany **INVITED**

Cells interact with their microenvironment by engaging membrane receptors with complementary partners at the surrounding matrix or at other neighbouring cells. These receptor complexes, often associated to cytoskeletal structures, allow exchange of biochemical and mechanical information. The ability to quantify this exchange is crucial for our understanding of cellular behavioru and responses to external factors. Using model bionterfaces with optoregulated interaction possibilities, selective membrane receptors in living cells can be addressed in situ, i.e. on a sensor surface, while quantifying specific cellular responses. Light-regulated tools to apply and sense cell biochemical and mechanical interactions will be presented.

Author Index

Bold page numbers indicate presenter

— A —
Alfonso Garcia, A: IPF+AS+BI+MN-TuM-3, **5**
Alsharif, N: BI+AS+IPF+NS-TuA-10, **8**
Ark, F: BI+AS+IPF+MN-MoA-10, 3
— B —
Baio, J: BI+AS+IPF+MN-MoA-3, **2**
Bartl, J: BI+AS+IPF+NS-TuA-3, **7**
Bednar, R: BI+AS+IPF+NS-TuA-9, **8**
Bhargava, R: IPF+AS+BI+MN-TuM-1, **5**
Black, F: IPF+AS+BI+NS-WeM-5, **9**
Boland, T: IPF+AS+BI+NS-MoM-5, **1**
Bonn, M: BI+AS+IPF+MN-MoA-5, 2
Brown, K: BI+AS+IPF+NS-TuA-10, 8
Bunch, J: IPF+AS+BI+MN-TuM-10, 5
— C —
Cao, Y: IPF+AS+BI+NS-WeM-3, 9
Castner, D: BI+AS+IPF+MN-MoA-4, 2
Cattani-Scholz, A: BI+AS+IPF+NS-TuA-3, 7
Cheng, F: BI+AS+IPF+NS-TuA-1, **7**; BI+AS+IPF+NS-TuA-8, 8
Cima, M: BI+AS+IPF+MN-MoA-1, **2**
Comi, T: IPF+AS+BI+MN-TuM-1, 5
Corrie, S: BI+AS+IPF+NS-TuA-2, 7; IPF+AS+BI+NS-WeM-10, **9**
Culbertson, R: BI+AS+IPF+MN-MoA-10, 3
— D —
Day, J: BI+AS+IPF+MN-MoA-10, 3
del Campo, A: IPF+AS+BI+NS-WeM-12, **9**
Dhayal, M: BI+AS+IPF+NS-TuA-7, **8**
— F —
Feng, Y: IPF+AS+BI+NS-WeM-3, 9
— G —
Gahlaut, S: BI+AS+IPF+NS-TuA-4, **7**
Gamble, L: BI+AS+IPF+MN-MoA-6, 3
Gaus, K: IPF+AS+BI+MN-TuM-5, **5**
Glass, J: IPF+AS+BI+NS-WeM-3, 9
Golbek, T: BI+AS+IPF+MN-MoA-3, 2
Graham, D: BI+AS+IPF+MN-MoA-6, 3
Gremmo, S: BI+AS+IPF+NS-TuA-3, 7
Gryka, M: IPF+AS+BI+MN-TuM-1, 5
— H —
He, W: BI+AS+IPF+NS-TuA-1, 7; BI+AS+IPF+NS-TuA-8, **8**
Herbots, N: BI+AS+IPF+MN-MoA-10, 3
Hood, M: BI+AS+IPF+MN-MoA-5, 2
Hsieh, J: BI+AS+IPF+NS-TuA-11, 8
Huynh, C: BI+AS+IPF+MN-MoA-9, **3**
— J —
Jansson, C: BI+AS+IPF+MN-MoA-8, 3
Johansson, P: BI+AS+IPF+MN-MoA-4, **2**
Johnson, C: BI+AS+IPF+MN-MoA-3, 2
— K —
Khan, A: BI+AS+IPF+NS-TuA-7, 8
Komorek, R: BI+AS+IPF+MN-MoA-8, 3
— L —
Lawton, T: BI+AS+IPF+NS-TuA-10, 8
Li, C: BI+AS+IPF+NS-TuA-11, 8
Lin, P: BI+AS+IPF+NS-TuA-11, **8**
Lu, H: BI+AS+IPF+MN-MoA-5, 2
Lutz, H: BI+AS+IPF+MN-MoA-5, 2
— M —
Mangus, M: BI+AS+IPF+MN-MoA-10, 3
Marcu, L: IPF+AS+BI+MN-TuM-3, 5
Marusak, K: IPF+AS+BI+NS-WeM-3, 9
Mehl, R: BI+AS+IPF+NS-TuA-9, 8
Muñoz-Espí, R: BI+AS+IPF+MN-MoA-5, 2
— N —
Narayan, S: BI+AS+IPF+MN-MoA-10, 3
Ngaboyamahina, E: IPF+AS+BI+NS-WeM-3, 9
Nicolas, J: IPF+AS+BI+MN-TuM-12, **5**
Notte, J: BI+AS+IPF+MN-MoA-9, 3
— O —
Oropeza, B: IPF+AS+BI+NS-MoM-5, 1
— Q —
Qu, J: BI+AS+IPF+NS-TuA-1, 7; BI+AS+IPF+NS-TuA-8, 8
— R —
Ratner, B: IPF+AS+BI+NS-MoM-1, **1**
Robinson, K: BI+AS+IPF+NS-TuA-2, **7**
Roeters, S: BI+AS+IPF+MN-MoA-5, 2
Rziga, G: BI+AS+IPF+NS-TuA-3, 7
— S —
Salditt, T: IPF+AS+BI+MN-TuM-12, 5
Scarbolo, P: BI+AS+IPF+NS-TuA-3, 7
Schäfer, A: BI+AS+IPF+MN-MoA-5, 2
Selmi, L: BI+AS+IPF+NS-TuA-3, 7
Sharan, C: BI+AS+IPF+NS-TuA-4, 7
Singh, J: BI+AS+IPF+NS-TuA-4, 7
Solis, L: IPF+AS+BI+NS-MoM-5, 1
Spatz, J: IPF+AS+BI+NS-MoM-3, **1**
Steven, R: IPF+AS+BI+MN-TuM-10, **5**
Stutzmann, M: BI+AS+IPF+NS-TuA-3, 7
Sun, B: BI+AS+IPF+NS-TuA-1, 7; BI+AS+IPF+NS-TuA-8, 8
— T —
Taylor, M: BI+AS+IPF+MN-MoA-6, **3**
Thinakaran, H: BI+AS+IPF+MN-MoA-10, **3**
Thurecht, K: BI+AS+IPF+NS-TuA-2, 7
Tornow, M: BI+AS+IPF+NS-TuA-3, 7
— U —
Uzarski, J: BI+AS+IPF+NS-TuA-10, 8
— V —
Vishwakarma, S: BI+AS+IPF+NS-TuA-7, 8
— W —
Wang, H: BI+AS+IPF+NS-TuA-1, 7
Wei, D: BI+AS+IPF+MN-MoA-9, 3
Weidner, T: BI+AS+IPF+MN-MoA-3, 2; BI+AS+IPF+MN-MoA-5, **2**
Wilkens, B: BI+AS+IPF+MN-MoA-10, 3
— Y —
Yanez, M: IPF+AS+BI+NS-MoM-5, 1
Yoo, J: IPF+AS+BI+NS-MoM-10, **1**
You, L: IPF+AS+BI+NS-WeM-3, 9
Yu, X: BI+AS+IPF+MN-MoA-8, **3**
— Z —
Zauscher, S: IPF+AS+BI+NS-WeM-3, **9**
Zheng, Y: IPF+AS+BI+NS-MoM-8, **1**
Zhu, C: BI+AS+IPF+NS-TuA-8, 8
Zhu, Z: BI+AS+IPF+MN-MoA-8, 3