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-TuM1 Chemical Imaging as a Tool to asses Molecular and Morphologic Content in Natural Tissues and Fabricated Models**, *R. Bhargava, T. Comi, M. Gryka,* ***Kevin Lee Yeh,*** 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-TuM3 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-TuM5 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-TuM10 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-TuM12 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

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