Biomaterial Surfaces & Interfaces
Room Milo - Session BI-TuM

Bioimaging & Bionanotechnology

Moderator: Sally L. McArthur, Swinburne University, Australia

8:00am **BI-TuM1 Gas Cluster Ion Beams for Improved Biological Imaging with ToF-SIMS**, ***John Fletcher,*** University of Gothenburg, Göteborg, Sweden

The quest for improved molecular signal levels in imaging secondary ion mass spectrometry has been a long one. As the nature of the primary ion beam used to sputter the surface has a direct influence on the species detected there has been a long history of research in this area. The latest ion beam technology of SIMS is based on gas cluster ions, introduced by Matsuo and co-workers. These beams are routinely used for sample etching and cleaning in SIMS and XPS instruments but are not normally employed as analysis beams in SIMS instruments due to challenges associated with fast pulsing, focusing and low analyte ionisation efficiencies.

The use of a higher energy (40 keV) gas cluster ion beam (GCIB) on a J105 ToF-SIMS instrument (Ionoptika Ltd), where fast beam pulsing is not required for good spectral quality, offers great benefits for biological analysis.

Comparison of signal levels on rodent brain between the 40 keV GCIB and equivalent energy C_{60} shows a 30-50× increase in secondary ion yield for intact lipids while spot sizes of approximately 2 µm have been achieved.

The application of this system for the imaging of animal and human tissue samples in cardiovascular and breast cancer research will be presented. Lipid changes following surgically induced infarction in mouse hearts have been imaged with distinct differences detected between the infarcted and healthy regions of the tissue along with specific lipid signals associated with the interface between the 2 regions. Breast cancer biopsy tissue has been imaged and the lipid distributions studied in the tumour and surrounding stroma. Changes in the abundance of lipids arising from modification of dietary fatty acids versus de novo synthesised lipids shed new light onto lipogenesis processes in the tumour microenvironment.

8:20am **BI-TuM2 ToF-SIMS Imaging for Nano-Bio Applications**, ***Tae G. Lee,*** Korea Research Instutue of Standards and Science (KRISS), Republic of Korea **INVITED**

Although the collisional cascade process in ToF-SIMS is unable to produce secondary ions with a molecular weight of over m/z 2,000 without the use of noble metals or special matrixes, time-of-flight secondary ion mass spectrometry (TOF-SIMS) imaging is a powerful technique for producing chemical images of small biomolecules (ex. metabolites, lipids, peptides) “as received” because of its high molecular specificity, high surface sensitivity, and submicron spatial resolution. For large biomolecules, matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been used for molecular identification of proteins in the discovery of disease-related biomarkers as a key platform technique in proteomics.

For this talk, I will show that the label-free ToF-SIMS imaging technique can be a platform technology for characterization of organic-conjugated nanoparticles, disease diagnosis and drug screening. In addition, I will discuss the potential capability of Ar-cluster SIMS to study omics, particularly proteomics and lipidomics for brain studies.

9:00am **BI-TuM4 Label-Free Imaging of Biological Tissue with Micron Spatial and 240k Mass Resolution using a New Sims Hybrid Mass Analyzer**, ***Nathan Havercroft,*** ION-TOF USA, Inc.*; A. Pirkl, R. Moellers, H. Arlinghaus, F. Kollmer, E. Niehuis,* ION-TOF GmbH, Germany*; A. Makarov, S. Horning,* Thermo Fisher Scientific, Germany*; M.K. Passarelli, R. Havelund, P. Rakowska, A. Race, A.G. Shard,* National Physical Laboratory, UK*; A. West, P.S. Marshall, C.F. Newman,* GlaxoSmithKline, UK*; M.R. Alexander,* University of Nottingham, UK*; C.T. Dollery,* GlaxoSmithKline, UK*; I.S. Gilmore,* National Physical Laboratory, UK

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is an established, highly sensitive analytical technique for mass spectrometry (MS) imaging applications with a lateral resolution below 100 nm. Application of this technique for the localization of drugs and their metabolites in drug-doped cells could be used to find regions in which a pharmaceutical compound accumulates. This information would be extremely helpful for selection of possible drug candidates in pre-clinical studies, thereby reducing the development costs for new pharmaceutical products. Furthermore, surveying biologically relevant molecules, such as lipids, in tissue can give valuable information on the molecular fundamentals of diseases and the effects of treatments. However, in complex biological samples identification of unknown compounds can be hampered by mass interferences and a high number of possible assignments for a single mass peak. In order to overcome these limitations, the 3D nanoSIMS project [1] is developing a revolutionary new SIMS instrument that combines the high lateral resolution and speed associated with TOF-SIMS (TOF.SIMS 5, ION-TOF GmbH, Muenster, Germany) with the high mass resolution and high mass accuracy of an orbital trapping mass analyzer (QExactive™ HF [2], Thermo Fisher Scientific™, Bremen, Germany). The instrument is equipped with a newly developed gas cluster ion beam column which provides the capability to image with a lateral resolution down to the micron level with minimum sub-surface damage. In this contribution we will report about results obtained from different biological application areas such as tissue imaging, lipidomics, and single cell analysis. From coronal mouse brain tissue slices, 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, which has not been possible before.

Analyzing NR8383 cells, 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.

Furthermore the MS/MS capability of the QExactive Instrument is used to confirm proposed assignments on tissue and from single cells.

[1] The 3D nanoSIMS project, http://www.npl.co.uk/news/3d-nanosims-label-free-molecular-imaging

[2] Scheltema, et al. Mol Cell Proteomics (2014).

9:20am **BI-TuM5 Advancing our Understanding of Tumor Biology with Imaging Time-Of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)**, ***Lara Gamble,*** *D.J. Graham, B. Bluestein,* University of Washington, USA

Imaging time-of-flight secondary ion mass spectrometry (ToF-SIMS) can provide images of tissues with chemical and molecular specificity. These chemically specific images can improve our understanding of biological processes. Our current research has utilized this technique to map the chemical changes in the composition and distribution of metabolic related molecules within a tumor and the tumor microenvironment in order to study tumor development and progression in inducible and regressible mouse pancreatic β-cell neoplasia. Using ToF-SIMS, the chemistry of tumor microenvironments and lipid metabolomics relationship to cancer and tissue can be visualized on a cellular and sub-cellular level. Samples mounted on ITO substrates can be analyzed with ToF-SIMS and directly correlated with immunohistochemical and/or H&E images taken on the same sample. Imaging principal component analysis (PCA) is used to identify chemical regions that correlate with the tumor and the surrounding tumor environment. Preliminary results using PCA analysis of ToF-SIMS image data easily separate the tumor chemistry from the surrounding tissue within the first principal component. Differences in chemistry between the tumor and surrounding tissue suggest a preferential uptake of fatty acids 18:3, 18:2 within the tumor. The data shows an absence of Mg^+ within the islet tumor and small, higher signal regions on the periphery of the tumor that correlate with increased CN^-, CNO^-, $C_7H_{10}O^+$, and Fe^+ ToF-SIMS peaks. This work demonstrates the high resolution capability of ToF-SIMS as the data clearly reveals intratumor chemical heterogeneity as localized high intensity regions for specific chemical signatures. It also highlights the utility of acquiring ToF-SIMS images and traditional H&E images on the same sample.

9:40am **BI-TuM6 Multi-stain Live Confocal Microscopy of *Balanus Amphitrite* Provides New Insight on Interfacial Processes**, ***Kenan Fears,*** *C. So,* US Naval Research Laboratory, USA*; B. Orihuela, D. Rittschof,* Duke University, USA*; K. Wahl,* US Naval Research Laboratory, USA

The adhesion of hard foulers (e.g., barnacles and tubeworms) has plagued the maritime community for as long as mankind has been setting sail. Since the biological processes responsible for adhesion occur at buried interfaces, elucidating the mechanisms by which foulers adhere is

challenging. Through the use of multiple fluorescent probes, peptides, and antibodies, we have been able to discern an unprecedented level of detail about biological processes that occur at the interface between acorn barnacles (Balanus Amphitrite) and the underlying substratum during the barnacle growth cycle. Barnacles secrete a lipidaceous substance around the outside of their shell, prior to expansion that dislodges microorganisms and biofilms to present a cleaned surface. During molting, epithelia cells build a new interfacial cuticular layer, which becomes autofluorescent as it is sclerotized, above the existing cuticle whose degradation coincides with the exuviation of the main body's cuticle. Rather than being directly secreted onto the substrate, nanostructured barnacle cement accumulates in the space in between the new and old cuticle. As the barnacle expands, the cuticular layers are stretched and pulled around the outside of the side plate. The strain causes the old cuticle to randomly tear, allowing the new cuticle to deposit cement into the interface as it is dragged across the substrate. Furthermore, antibody staining allowed us to spatially and temporally identify where different cement proteins are presenet. These results illustrate that the methodologies we have developed to break down and analyze barnacle cement collection are yielding a more accurate representation of the proteins at the buried interface, and providing insight on their roles which will lead to improved strategies to both combat and mimic barnacle adhesives.

10:20am **BI-TuM8 Nanodiamonds for Targeted Biolabeling**, ***Olga Shimoni***, *K. Bray, L. Cheung, I. Aharonovich,* University of Technology Sydney, Australia

In the last decade, nanodiamonds (NDs) have attracted much interest from the biological community as the ultimate agent for biomedical applications, such as biomarkers, drug and gene delivery and biocatalysts, owing to their chemical inertness, biocompatibility, prolonged photostability and negligible toxicity. Their fluorescence emanates from point defects (centres) that possess an unprecedented photostability and exhibit visible emission at room temperature. One of the most well-known centres is nitrogen-vacancy (NV) defects, mostly because of its potential use in quantum computer and nanomagnetometry applications. Here, we will demonstrate the utilisation of commercially available NDs with NV centres and in-house fabricated NDs with silicon-vacancy (SiV) defects for application in bio-imaging. We will discuss their optical properties, luminescence differences and opportunities for fluorescence enhancement. An additional advantage of using NDs in bio-imaging is that they are purely made of carbon, and carbon can be readily modified with functional groups to attach biomolecules using standard organic chemistry procedures. Therefore, we demonstrate surface functionalisation of NDs to achieve selective intracellular targeting. In summary, our results bring new advancement in fabrication and utilisation of NDs as targeted biomarkers.

10:40am **BI-TuM9 Encapsulation of Selected Natural Novel Nutraceutical Biomolecules**, ***Selim Kermasha***, McGill University, Canada; *S. Karboune,* McGill Universit, Canada

Although there is an increasing interest in the development of novel natural nutraceuticals, it is important to ensure their preservation and delivery by their encapsulation, a technology that is gaining popularity in the food industry. The encapsulation of selected nutraceuticals, including enzymatically-synthesized phenolic lipids and self-assembly polymers of proteins and polysaccharides, was carried out. Our research group succeeded in the production of novel biomolecules of high nutritional value and antioxidant capacity, phenolic lipids (PLs), by a biotechnological process involving the esterification of selected phenolic acid models and endogenous edible oils. The encapsulation of PLs was carried out by the development of a process to yield gelatin-gum Arabic multinuclear microcapsules, via complex coacervation. The overall experimental findings indicated that the microencapsulation of PLs was effective for preventing their oxidation and hence by maintaining their antioxidant potential. On the other hand, the self-assembly of selected proteins (patatin and lysozyme)/polysaccharides (galactan, gum Arabic and xanthan gum)-based nanoparticles was investigated. The results indicated that the nanoparticles have spherical shape and their sizes were dependant on the pH and the molar ratio of protein to polysaccharide. Through the zeta (ζ) potential measurements, the formation mechanism of amphoteric patatin/xanthan gum and lysozyme/galactan nanoparticles was illustrated.

11:00am **BI-TuM10 Sequential Drug Release by pH/redox Dual Responsive Non-covalent Polymer Gatekeepers in Hollow Mesoporous Silica Nanoparticle**, ***Ja-Hyoung Ryu***, Ulsan National Institute of Science and Technology, Republic of Korea

Nanoscopic delivery vehicles capable of encapsulating drug molecules and releasing them in response to

external stimuli are of great interest due to implications in therapeutic applications. Sequential drug delivery with dual

stimulus responsive nanotherapeutics is highly desirable for disease specific treatment in cancer therapy with

minimized adverse effects. In addition to this, on-demand therapy received considerable attention among the

treatment techniques. Herein, we present the design of robust, new and simple pH dependent charge conversional

non-covalent polymer gatekeepers technique by preparing the hydrophilic and hydrophobic drug loading at high

capacity and improved encapsulation stability in hollow mesoporous container for target specific cellular uptake for

cancer treatment. The di-isopropyl methacrylate functionalized monomer facilitates the fast cellular uptake at acidic

environment of cancer cells and allows the on-demand release of hydrophillic drug at acidic pH of endosomes upon

protonation. Pyridine disulfide facilitates the strong encapsulation of loaded cargo upon crosslinking by thiol-disulfide

exchange and releases the cargo upon exposure with increased intracellular glutathione concentration. The codelivery

of the multi-drugs in single carrier enables a synergistic chemotherapeutic effect. Based on this new design, a

wide range of sequential and synergistic therapy can be achieved to satisfy varied clinical requirements.

11:20am **BI-TuM11 Development Of Biometric Identification Technique Of High Reliability Based On Atomic Force Microscopy**, ***Vlad Ageev***, Biomaging and Bionanaotechology, Russia

In this paper a method of biometric identification with high reliability based on measuring the elastic properties of the skin of a human finger while scanning his finger prints is presented. This method is shown to allow with a high degree of veracity to distinguish the skin from the inorganic materials used to create the fingerprint. It is found that the elasticity of the skin varies at 15% with increasing interval between the cut and measurement of the skin from 5 to 30 minutes. The elasticity of the skin also depends on the age of the person and is 60,2 ± 4,2 and 42,4 ± 2,6 kPa to 20 and 40 years, respectively. These dependencies can be used for creating additional levels of protection of biometric identification method and preventing such methods of its compromетation as the use of moulds and pre-made cuts of skin. The results can be used in the development of biometric identification systems with a high level of protection that verifies either the fingerprint pattern of skin of human finger or its elasticity.

Nanotechnology; biometrics; biometric identification; skin; elasticity; atomic force microscopy

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