Biomaterial Interfaces
Room 117 - Session BI1-TuM

Characterization of Biological and Biomaterial Surfaces I: Celebration of Stephanie Allen

Moderators: Morgan Hawker, California State University, Fresno, **Sapun Parekh**, University of Texas at Austin

8:00am **BI1-TuM-1 Biointerfacial Characterisation of Implanted Medical Devices with OrbiSIMS***, **Morgan Alexander,*** University of Nottingham, UK

The 3DOrbiSIMS hyphenation of ToF SIMS with an OrbiTrap™ makes meaningful analysis the molecules in complex biological samples and bio-interfaces formed on materials feasible. [1,2] Critically, the mass resolving power and mass accuracy has rendered routine peak assignment with deviations below 2 ppm. The large spectral data files with thousands of peaks that arise from biological samples requires automated untargeted analysis to make the most of this information. These have been enabled by the methodology for molecular formula prediction (MFP) assignment adapted to SIMS by Edney et al. [3]

I will illustrate how this enables us to investigate the bio-interface for implanted medical devices, to shed light on their failure mechanisms. The importance of the lipids and other metabolites is revealed in the analysis of tissue sections. Subsequent analysis of the bio interfacial deposit at the surface of extracted devices sheds light on the complexity of this process. [4,5] Understanding medical implant fibrosis by biointerfacial OrbiSIMS analysis [Bin Sabri unpublished]

References

1. Mass spectrometry and informatics: distribution of molecules in the PubChem database and general requirements for mass accuracy in surface analysis. FM Green, IS Gilmore, MP Seah (2011) *Analytical Chemistry*.
2. The 3D OrbiSIMS: Label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power. Passarelli et al. (2017) *Nature Methods*.
3. Molecular formula prediction for chemical filtering of 3D OrbiSIMS Datasets. Edney et al (2022) *Anal Chem.*
4. Single-cell metabolic profiling of macrophages using 3D OrbiSIMS: Correlations with phenotype. Suvannapruk et al. (2022) *Anal Chem*
5. Spatially resolved molecular analysis of host response to medical device implantation using the 3D OrbiSIMS highlights a critical role for lipids. Suvannapruk et al. (2024) *Advanced Science*.

8:15am **BI1-TuM-2 Insights into the Chemistry of Wheat Leaves and their Uptake of Agrochemicals using OrbiSIMS***, M. Khan,* University of Nottingham, UK*; C. Whitehouse, T. Powell,* Syngenta, UK*; C. Roberts,* ***David Scurr,*** University of Nottingham, UK

The estimated size of the global agrochemicals market in 2022 amounted to USD 227.9 billion with a projected increase to USD 234.27 billion in 2023 [Market Analysis Report 2018-2022]. The notable increase in agrochemical usage observed worldwide can be attributed to the economic benefits that to farmers through the safeguarding of crops against invasive species, including the improvement in quality and quantity of harvests. Limited knowledge of the *in-situ* chemical composition of wheat leaves and the permeation mechanisms of pesticides into skin and leaf tissues constrains research and development of new products.

OrbiSIMS has been recently demonstrated as a powerful tool for skin research, providing label-free insight into the 3D permeation profiles of endogenous and exogenous compounds. Previous work [Starr et. al., *PNAS*, 2022] investigated the molecular composition of the *stratum corneum* and tracking the permeation of an active agent. Our study expands the use of OrbiSIMS to investigate the native chemistry of wheat leaves, particularly the plant cuticle as the primary diffusion barrier. The study reveals the distribution of a fungicide formulation through both wheat leaves and skin, offering insights into its diffusion in relevant biological matrices.

The molecular architecture of wheat leaves was first probed, with a focus on the cuticle. *In-situ* analysis provided novel insights into the localisation of endogenous species, including fatty acids, aldehydes, phospholipids, flavones and vitamins. Depth profiling revealed depth-dependent variations in leaf structure, with fatty acids and aldehydes associated with the cuticle and epicuticular waxes exhibiting a prominent concentration at the leaf surface. Conversely, flavones and vitamins were predominant in the epidermis.

Exogenous compounds were identified in skin and wheat leaves, alongside endogenous species. The investigation focused on the impact of exposure time and concentration on agrochemical permeation across skin and wheat leaves. *In-situ* analysis provided the detection and tracking of the entire formulation, even at 100 ppm. Cyproconazol exhibited enhanced permeation with prolonged exposure time and higher concentrations in both matrices. Co-formulants showed varied localization patterns, with carrier solvents resembling the permeation of cyproconazole and emulsifiers remained primarily at the surface.

8:30am **BI1-TuM-3 Imaging 3D Cell Culture Systems***, **Sally McArthur,*** Deakin University, Australia

Imaging three dimensional cell and tissue systems is central to our fundamental understanding of tissue engineered materials. We need to be able to look at the cell morphology, scaffold architecture and their specific interactions. By combining a range of tools we have explored how biomaterials and cells interact in 3D and the reproducibility of 3D cell culture systems. This talk is submitted as part of the celebration of Prof Stephanie Allen's career.

8:45am **BI1-TuM-4 SIMS for Label-Free in situ Analysis of Glycosaminoglycans***, **Li Jennifer Lu,*** University of Nottingham, UK*; J. Hippensteel,* University of Colorado - Anschutz Medical Center*; K. Grobe,* University of Münster, Germany*; C. Gorzelanny,* University Medical Center Hamburg - Eppendorf, Germany*; A. Kotowska, D. Scurr, A. Hook,* University of Nottingham, UK

Glycosaminoglycans (GAGs) are linear polysaccharide chains with many varied roles in physiology, including embryonic patterning and modulation of blood vessel permeability. Despite their biological importance, their *in-situ* analysis is limited by a lack of analytical tools with which to study their complex structure. Here we present the development of secondary ion mass spectrometry (SIMS) for *in-situ* GAG analysis [1], allowing for simultaneous spatial and compositional analysis. Initially, a list of characteristic ions for different GAG types was identified using high mass resolution analysis using an Orbi-trap mass analyser of a library of reference GAGs. These GAG-derived ions were validated using a range of biosynthetic enzyme knockout cellular models. This approach has been used to spatially assess the distribution of varied GAG types within complex tissues, including a sepsis model and to explore embryogenesis within *Drosophila*. Additionally, the depth profiling capability of SIMS enables 3D imaging of GAG ions within samples. This demonstrated ToF-SIMS as a powerful analytical tool to spatially analyse (at near optical resolution) GAG type and composition within a single analysis across multiple biological sample types.

References

1. Hook, A.L., Hogwood, J., Gray, E. et al. High sensitivity analysis of nanogram quantities of glycosaminoglycans using ToF-SIMS. Commun Chem 4, 67 (2021).

9:00am **BI1-TuM-5 Tribochemical Nanolithography – Fast, Simple Biomolecular Nanopatterning with 23 nm Resolution at Speeds of up to 1 mm s-1***, O. Siles-Brugge, C. Ma, A. Meijer,* ***Graham Leggett*****,** University of Sheffield, UK

Films formed by the adsorption of (methoxyheptaethylene glycol) nitrophenylethoxycarbonyl-protected aminopropyltriethoxysilane (OEG-NPEOC-APTES) on silica are highly resistant to the adsorption of proteins. On exposure to UV light, the photocleavable protecting group is removed allowing the immobilization of biomolecules.

We have discovered that the same result can be achieved using an AFM probe at a load of ca. 100 nN in the absence of UV light. A FWHM of 23 nm can be achieved at a writing rate of 1 mm s^{-1}. The FWHM increases with load, reaching 90 nm at a load of 10 μN. At larger loads than this an abrupt transition occurs to a regime dominated by mechanical abrasion, yielding broader features. However, for control films that do not contain photo-removable protecting groups, lithographic modification was not observed at loads below 10 μN.

We hypothesize that at low loads the AFM probe causes selective cleavage of the same C-N bond in the carbamate group that is cleaved during UV irradiation. Consistent with this, we found that patterned surfaces can be derivatized with nitrilotriacetic acid (NTA) functional groups, enabling coupling of His-tagged green fluorescent protein (GFP) to the surface.

Confocal fluorescence microscopy confirms that GFP attaches to nanolines, but is released when the samples are treated with imidazole, which disrupts the interaction between NTA and the His tag on the protein, consistent with site-specific binding.

The effect of compression on the nitrophenyl protecting group was explored using density functional theory (DFT). Our results indicate that compression of the nitrophenyl group causes substantial changes in its electronic structure. In particular, the energy of the main energetic barrier in the photodeprotection scheme, the initial S_0 to S_1 transition, is greatly reduced, so that deprotection may occur at near IR wavelengths. Hence, application of the AFM probe facilitates deprotection by low energy photons, while UV photons are required in the absence of a mechanical deformation.

The methodology may also be applied to the fabrication of polymer nanostructures. Tribochemical nanolithography of nitrophenylpropyloxyoxycarbonyl protected aminopropyl triethoxysilane (NPPOC-APTES) films yields amine-functionalised nanolines that are functionalized with bromine initiators and used to grow surface-grafted polymer brushes. Polymer chains grafted to the smallest nanolines are collapsed, because they have a high free volume and because adsorption to the surface is energetically favourable. However, as wider structures are formed, the chains repel each other and begin to swell away from the surface.

9:15am **BI1-TuM-6 Nanoprobe X-Ray Fluorescence Analysis of Frozen-Hydrated Biological Samples - from 2D to 3D***, **Axel Rosenhahn**, C. Rumancev, L. Jusifagic, A. Gräfenstein,* Ruhr University Bochum, Germany

The accumulation of metals and the homeostasis of ions in biological cells and tissue is of fundamental relevance for a wide range of environmental, biological, and medical processes. Synchrotron-based nanoprobe X-ray fluorescence analysis provides a unique combination of metal analysis with high spatial resolution, a high penetration depth, and high sensitivity down to trace concentrations. In the last years we developed several endstations for the analysis of cryogenically prepared biological samples at the P06 beamline at Petra III. Cryopreservation is the gold standard if cells are meant to be analyzed in a preserved state that is as close as possible to their natural, hydrated state. In particular for highly soluble ions, such as potassium, cryopreservation is the only way to obtain accurate concentrations. The new technique has been used to analyze the stress response of cells to the presence of Huntingtin aggregates, which are currently hypothesized to be responsible for the consequences of the corresponding disease. Also, the intracellular distribution of different metal-based cytostatic compounds has been analyzed and compared to the cellular stress response as reflected by changes in the intracellular potassium level. In addition to the 2D imaging experiments, a new tomography setup has been developed that allows cross-sectional imaging of biological samples to image metal distributions. A novel self-absorption correction during the tomographic reconstruction has been implemented that compensates artefacts especially for light elements due to the limited photon-escape depth.

9:30am **BI1-TuM-7 Harnessing Plasmon-Enhanced Fluorescence for Ultrasensitive and Minimally-Invasive Bio-Diagnostics***, **Srikanth Singamaneni**,* Washington University in St. Louis **INVITED**

Detection, imaging, and quantification of low-abundant biomolecules within biological fluids, cells, and tissues is of fundamental importance but remains extremely challenging in biomedical research as well as clinical diagnostics.We have designed and synthesized an ultrabright fluorescent nanoconstruct, termed “plasmonic-fluor”, as an “add-on” bio-label to dramatically improve the signal-to-noise ratio of a wide variety of existing fluorescence bioassays without altering or complicating the conventional assay workflow or read-out devices.We demonstrate that these novel nanoconstructs can be readily utilized in a broad range of bioanalytical methods, including fluorophore-linked immunosorbent assays, multiplexed bead-based immunoassays, lateral flow assays, immuno-microarrays, flow cytometry, and immunocytochemistry, to attain more than 1000-fold improvement in the limit-of-detection and dynamic range.Harnessing plasmonic-fluors, we also demonstrate minimally-invasive and ultrasensitive quantification of target protein biomarkers in interstitial fluid through microneedle-assisted *in vivo* sampling and subsequent on-needle analysis.With the microneedle patch, we demonstrate minimally-invasive evaluation of cocaine vaccine efficiency and longitudinal monitoring of inflammatory biomarker levels in mice.

Author Index

Bold page numbers indicate presenter

— A —
Alexander, Morgan: BI1-TuM-1, **1**
— G —
Gorzelanny, Christian: BI1-TuM-4, 1
Gräfenstein, Andreas: BI1-TuM-6, 2
Grobe, Kay: BI1-TuM-4, 1
— H —
Hippensteel, Joseph: BI1-TuM-4, 1
Hook, Andrew L.: BI1-TuM-4, 1
— J —
Jusifagic, Lejla: BI1-TuM-6, 2
— K —
Khan, Mohammed: BI1-TuM-2, 1
Kotowska, Anna M.: BI1-TuM-4, 1
— L —
Leggett, Graham: BI1-TuM-5, **1**
Lu, Li Jennifer: BI1-TuM-4, **1**
— M —
Ma, Camery: BI1-TuM-5, 1
McArthur, Sally: BI1-TuM-3, **1**
Meijer, Anthony: BI1-TuM-5, 1
— P —
Powell, Tim: BI1-TuM-2, 1
— R —
Roberts, Clive: BI1-TuM-2, 1
Rosenhahn, Axel: BI1-TuM-6, **2**
Rumancev, Christoph: BI1-TuM-6, 2
— S —
Scurr, David: BI1-TuM-2, **1**
Scurr, David J.: BI1-TuM-4, 1
Siles-Brugge, Oscar: BI1-TuM-5, 1
Singamaneni, Srikanth: BI1-TuM-7, **2**
— W —
Whitehouse, Conor: BI1-TuM-2, 1