Tandem MS Focus Topic
Room 5 & 6 - Session TM+AS-MoM

New Instrumentation Featuring Tandem MS

Moderators: Chris Anderton, Pacific Northwest National Laboratory, Daniel Graham, University of Washington

8:20am **TM+AS-MoM-1 *In Situ* MS/MS Analysis on Biological Samples using Imaging Secondary Ion Mass Spectrometry (SIMS)*, Hua Tian,*** Pennsylvania State University **INVITED**

SIMS imaging allows characterization of biomaterials with high lateral resolution. The method has not, however, yet gained popularity within the biological community. One reason is the need for MS/MS analysis due to isobaric interferences associated with the complex composition of biomaterials. Although MS/MS has been routine in imaging mass spectrometry (IMS) with matrix assisted laser desorption ionization (MALDI), it is rarely incorporated with SIMS imaging. Until recently, only a few SIMS instruments had the capability of tandem or parallel MS/MS imaging, for example the J105 3D Chemical Imager, PHI nanoTOF II and IonToF Orbitrap Hybrid [1-3]. The identification of lipids and metabolites in various biosystems, such as Drosophila brain section[4], Zebra Finch brain section, Zebrafish whole body section[1] and bacteria [2], has been reported so far. The precise precursor selection, high lateral resolution and high energy collisional fragmentation are the must-have for the design of new instrumentation for MS/MS capability. Gas cluster ion source (GCIB) is also a necessity for generating sufficient precursor ions especially at extended mass range of SIMS spectra, allowing for a much greater variety of biomolecule studies. Here, we present a review of the current state of MS/MS in SIMS, and illustrate the power of this technique using a hybrid mass spectrometer that employs shaped field bunching for injection into the collision cell. The possibility to utilize laser-induced photo-fragmentation in this instrument is also discussed.

1. Fisher, G. L.; Bruinen, A. L.; Ogrinc Potočnik, N.; Hammond, J. S.; Bryan, S. R.; Larson, P. E.; Heeren, R. M. A., A New Method and Mass Spectrometer Design for TOF-SIMS Parallel Imaging MS/MS. *Analytical Chemistry* **2016,***88* (12), 6433-6440.

2. Wehrli, P. M.; Lindberg, E.; Angerer, T. B.; Wold, A. E.; Gottfries, J.; Fletcher, J. S., Maximising the potential for bacterial phenotyping using time-of-flight secondary ion mass spectrometry with multivariate analysis and Tandem Mass Spectrometry. *Surf Interface Anal* **2014,***46*, 173-176.

3. Hybrid SIMS. https://www.iontof.com/hybrid-sims-ms-ms-organic-mass-spectrometry-surface-analysis.html (accessed April 28, 2017).

4. Phan, N. T. N.; Munem, M.; Ewing, A. G.; Fletcher, J. S., MS/MS analysis and imaging of lipids across Drosophila brain using secondary ion mass spectrometry. *Analytical and Bioanalytical Chemistry* **2017**, 1-10.

9:00am **TM+AS-MoM-3 Molecular Depth Profiling with a New Hybrid SIMS Instrument for Improved Molecular Identification using Tandem MS*, Alexander Pirkl****,*** *R Moellers, H Arlinghaus, J Zakel, D Rading, E Niehuis,* ION-TOF GmbH, Germany

The characterisation of organic layer systems is of increasing interest in many research areas. Since the application of large argon clusters as sputter species in SIMS, depth profiling of almost all organic materials has become feasible whilst retaining the intact molecular information during the profile.

However, molecular identification of unknown substances, e.g. contaminants, can be hampered by constraints in mass resolution and mass accuracy of a standard TOF analyser. To overcome this problem, we have developed a new Hybrid SIMS instrument, which uniquely combines all advantages of a state-of-the-art TOF-SIMS with the mass spectrometry performance of an Orbitrap mass analyzer (Q Exactive™ HF) [1]. The Q Exactive mass spectrometer provides a mass resolution of more than 240,000 @ m/z = 200, sub ppm mass accuracy, and fully integrated MS/MS capabilities that allow low energy collision induced fragmentation for structural analysis of complex molecules. All in all this dramatically increases the level of confidence for the SIMS analysis.

In this contribution, we will present the new instrument and discuss applications from various fields including organic electronics. We will demonstrate how the extremely high mass resolution of the Q Exactive mass spectrometer can be advantageously used to resolve mass interferences which cannot be separated in a standard TOF-SIMS instrument. We will also show examples of structural analysis using the high-performance MS/MS capabilities and discuss the new possibilities of the unique TOF / Q Exactive mass spectrometer combination.

[1] Passarelli et al, The 3D OrbiSIMS – A new Method for Label-Free Metabolic Imaging with Sub-cellular Lateral Resolution and High Mass Resolution, submitted 2017.

9:20am **TM+AS-MoM-4 Spatially-resolved Tandem Mass Spectrometry Increases Molecular Confidence in a Multimodal Mass Spectrometry Imaging Investigation of a Tripartite Plant-fungus-cyanobacteria Interaction*, Dušan Veličković,*** Pacific Northwest National Laboratory*; A Carrell,* Duke University*; R Chu,* Pacific Northwest National Laboratory*; D Pelletier,* Oak Ridge National Laboratory*; L Paša-Tolić,* Pacific Northwest National Laboratory*; D Weston,* Oak Ridge National Laboratory*; C Anderton,* Pacific Northwest National Laboratory

Plant microbiomes represent a complex mix of interacting species with diverse physiologies and phylogenetic origins. Their functional outcomes are critical to biogeochemical cycles, yet measuring molecular (e.g., metabolite) exchange among interacting species is a major technical challenge. Traditional bulk metabolomic technologies are often limited in their ability to distinguish between molecules that remain localized within microbes and exuded molecules that are in proximity, thus often disregarding the multifaceted chemical exchange within and between interacting species. Mass spectrometry imaging (MSI) methodologies have been recently adopted to visualize the flow of metabolites produced by agar-supported microbial colonies. Several ionization modalities are suitable for MSI of microbial communities, with matrix-assisted laser desorption/ionization (MALDI) being most commonly used. When coupled with ultra-high resolution mass analyzers (e.g., Fourier transform ion cyclotron resonance mass spectrometers; FTICR-MS), these imaging sources offer the high mass resolution and accuracy needed for putative identification of metabolites in individual pixels in the image. However, orthogonal methodologies (e.g., tandem MS) are often required for confident metabolite identification.

Herein, we explored the interactions within a tripartite system of moss, cyanobacteria, and fungus using a multimodal imaging strategy, which employs liquid extraction surface analysis (LESA) tandem MSI to examine previously MALDI imaged samples. This method improved exometabolite identification confidence by preserving spatial dimensionality in the tandem MS experiment. Specifically, we found the combination of these two imaging modalities generated very congruent mass spectral information, providing the link between highly accurate structural information offered by LESA and high spatial resolution attainable by MALDI. Finally, FTICR-based secondary ion mass spectrometry provided new insights into tripartite community using correlative fragment data (SIMS and LESA-MS/MS), while delivering higher lateral resolution MS images. These multimodal imaging results offer detail metabolic insights into a moss, cyanobacterium, and fungus in isolation and when in a tripartite symbiosis.

9:40am **TM+AS-MoM-5 The Biosynthesis of Protective Metabolites in Amazonian *Sextonia rubra* Revealed by 100 nm-Scale TOF-SIMS Tandem MS Imaging*, Gregory L. Fisher,*** Physical Electronics*; T Fu, D Touboul,* Institut de Chimie des Substances Naturelles, CNRS, France*; S Della-Negra,* Institut de Physique Nucléaire, CNRS, France*; E Houël, N Amusant, C Duplais,* Cirad, UMR EcoFoG, AgroParisTech, CNRS, INRA, France*; A Brunelle,* Institut de Chimie des Substances Naturelles, CNRS, France

We have explored the botanical synthesis of bioactive molecules in the wood of *S. rubra* (Figure 1) via TOF-SIMS Parallel Imaging MS/MS. This investigation is part of an effort to develop a new strategy for investigating natural product formation in relation to the secondary metabolite synthesis during heartwood formation. The TOF-TOF tandem mass spectrometer of the PHI *nanoTOF* II enabled, for the first time in this field of study, simultaneous surface screening of the botanical matrix chemistry by TOF-SIMS (MS^1) imaging and targeted identification of biosynthetic components by MS/MS (MS^2) imaging [1]. Imaging of molecules with unambiguous identification occurred in minutes without observable degradation of the specimen. Hence, the wood chemistry was broadly profiled while multiple tandem MS imaging analyses were performed for discovery.

The metabolites of rubrynolide and rubrenolide, having significant xylophage toxicity and antifungal properties [2], are produced in oil cells that are found in close proximity to both vessels and parenchyma cells. Moreover, there are thought to be several bio-molecular precursors en route to these bioactive metabolites. Our goal was to identify biosynthetic precursors, and to verify their coincidence with rubrynolide and

rubrenolide, via tandem MS imaging. We were able to demonstrate the presence of numerous precursors and to confirm or derive their structure using the tandem MS product ion spectrum, thus contributing in the exploration of natural product biosynthesis.

[1] (a) G.L. Fisher, A.L. Bruinen, N. Ogrinc Potočnik, J.S. Hammond, S.R. Bryan, P.E. Larson, R.M.A. Heeren, *Anal. Chem*. **2016**, DOI: 10.1021/acs.analchem.6b01022. (b) G.L. Fisher, J.S. Hammond, P.E. Larson, S.R. Bryan, R.M.A. Heeren in *SIMS XX Proceedings* (Ed.: D. Castner), Wiley, New Jersey, **2016**, DOI: 10.1116/1.4943568.

[2] (a) A.M.S. Rodriguez, P.N.E.T. Theodoro, V. Eparvier, C. Basset, M.R.R. Silva, J. Beauchêne, L.S. Espíndola, D. Stein, *J. Nat. Prod*. **2011**, DOI: 10.1021/np1001412. (b) A.M.S. Rodriguez, N. Amusant, J. Beauchêne, V. Eparvier, N. Lemenager, C. Baudasse, L.S. Espíndola, D. Stein, *Pest Manag. Sci*. **2011**, DOI: 10.1002/ps2167.

10:40am **TM+AS-MoM-8 Metabolite Annotation for Ultra-HR Imaging Mass Spectrometry: MS1 and Beyond***, **Theodore Alexandrov**,* European Molecular Biology Laboratory, Germany **INVITED**

Metabolite imaging mass spectrometry promises to localize small molecules, metabolites, and lipids in tissues, microbial and cell cultures, and to interpret them in the context of cellular heterogeneity. However, just until recently the molecular interpretation of the big data generated by this technique was hampered by the lack of bioinformatics for metabolite identification. We recently developed and implemented a bioinformatics approach that allowed us to identify hundreds of metabolites from hundreds of datasets from various biological systems. We will present how this big data mining approach helps extract molecular knowledge from terabytes of imaging mass spectrometry data, find the link between metabolism and disease, and picture metabolites across hundreds of datasets.

11:20am **TM+AS-MoM-10 Multivariate Analysis of combined ToF-SIMS and Orbitrap-SIMS data***, **Henrik Arlinghaus**, M Keenan, A Pirkl, R Moellers, E Niehuis,* ION-TOF GmbH, Germany

Advances in SIMS instrumentation, such as the advent of gas cluster ion sources, have greatly increased the analysis capabilities on organic samples, e.g. by reducing molecular fragmentation. However, the identification of molecules may still be limited by the mass resolution and mass accuracy of the analyzer. A Hybrid SIMS instrument[1], combining a ToF-SIMS mass analyzer and an Orbitrap™ mass analyzer (Q Exactive™ HF) has been developed in order to overcome these limitations, combining the high lateral and depth resolution and repetition rate of the ToF-SIMS analyzer with the high mass resolution, mass accuracy, and MS-MS capabilities of the Q Exactive HF analyzer (240,000 @ m/z = 200, sub ppm accuracy). This instrument generates a vast amount of data, rendering manual analysis of the full dataset impractical.

Multivariate analysis (MVA) may be used to reduce complex datasets to a small set of relevant factors, simplifying data interpretation. Established multivariate techniques, such as principal component analysis (PCA), have been used to analyze everything from a small set of inorganic spectra to complex three dimensional organic samples consisting of hundreds of millions of voxel spectra, such as OLEDs. These techniques are now routinely used for ToF-SIMS data analysis in many laboratories.

We will present results of multivariate analysis of datasets acquired using a Hybrid SIMS instrument, where we simultaneously analyzed both the ToF-SIMS and Orbitrap-SIMS data. This type of analysis presents unique challenges, such as contending with vastly different detector technologies and the corresponding differences in noise characteristics.

[1] Passarelli et al, The 3D OrbiSIMS – A new Method for Label-Free Metabolic Imaging with Sub-cellular Lateral Resolution and High Mass Resolution, submitted 2017.

Author Index

Bold page numbers indicate presenter