Thursday Morning, September 22, 2022

Dealing with Data and Interpretation
Room Great Lakes B - Session DI-ThM1

Data and Data Processing

Moderators: Christine Mahoney, Corning Research and Development Corporation, **Bonnie J. Tyler**, University of Münster

8:40am **DI-ThM1-1 Denoising of Tof-Sims Images via Inverse Maximum Signal Factors Analysis**, ***Bonnie J. Tyler***, *H. Arlinghaus,* University of Münster, Germany

One of the long-term objectives of ToF-SIMS research has been the high resolution 2D and 3D imaging of pharmaceuticals and biomolecules in tissues and biofilms at physiologically relevant concentrations.Although much progress has been made through advances in instrument design and development of cluster ion sources, the technique continues to be limited due to low signal-to-noise ratio for many important systems.Improving signal-to-noise, and thereby image contrast, is one of the key challenges needed to expand the useful applications of ToF-SIMS.Various multivariate analysis (MVA) methods have proven to be effective for improving image contrast in ToF-SIMS.However, the distribution of important but low intensity ions can be obscured in the MVA analysis leading to a loss of chemically specific information.In this work we propose inverse maximum signal factors (iMSF) denoising as an alternative approach to both denoising and multivariate analysis for ToF-SIMS imaging.This approach differs from the standard MVA techniques in that the output is denoised images for each original mass peak rather than the frequently difficult to interpret scores and loadings. Five tests have been developed to optimize and validate the resulting denoised images.The algorithm has been tested on a range of simulated data with different levels of noise, correlated noise, varying numbers of underlying components and non-linear effects.In the simulations, excellent correlation between the true images and the denoised images was observed for peaks with an original signal-to-noise ratio as low as 0.1 as long as there was sufficient intensity in the sum of selected peaks.Applications of this approach for 2D imaging of glycolipid accumulation in Fabry mouse kidney, 3D imaging of antibiotics in frozen/hydrated biofilms and MALDI-MSI imaging of mouse brain will be presented.A signal-to-noise improvement of as much as two orders-of-magnitude was achieved for very low intensity peaks.MSF denoising is a powerful addition to the suite of image processing techniques available for studying mass spectrometry images.

9:00am **DI-ThM1-3 High-Speed 3D ToF-SIMS Analysis of Unknown Samples**, *A. Bellew, N. Sano, A. Stickland, P. Blenkinsopp,* Ionoptika Ltd, UK*; K. McHardy,* ***Michal Ryszka,*** Ionoptika Ltd., UK

ToF SIMS allows for high-resolution 3D tomography, where each voxel contains a mass spectrum. With spatial resolutions of hundreds of nanometres and depth resolutions of just a handful of nanometres, we should expect ToF SIMS to be used in every analysis lab worldwide. It is not the case, of course.

Up to now, 3D ToF SIMS images were a “nice to have” - the cherry on top once the analysis was complete. Something for the journal cover or some pretty marketing material, but often no more than that. However, it is not for lack of desire but rather a lack of computing power. These authors believe 3D analysis should be part and parcel of the ToF SIMS workflow.

The J105 SIMS collects 200,000 bins per spectrum, up to 32 bits each. A good quality 3D image contains at least 128x128x128 pixels – more than 2 million voxels. This amounts to more than a terabyte of uncompressed data! Existing methods would require a high-powered server to load this data to make each mass image accessible to the user in a reasonable time frame.

We present here for the first time a new software tool for high-speed inspection and analysis of large 3D data sets that requires very little computing power and can operate on a mid-range laptop computer. Coupled with Ionoptika’s smart compression solution, Analyse3D can display 3D images of a single peak in mere seconds. All peaks in the data set may thus be imaged and compared in 3D quickly and easily.

We shall demonstrate the capabilities of this new tool on OLED devices, multi-layer coatings, ion implantation samples, and more.

All the features you expect from 2D analysis, but with the flexibility of 3D. Analyse3D™ completely changes the way we look at ToF SIMS data.

9:20am **DI-ThM1-5 Towards Comprehensive Analysis of Complex Biological Samples in 3D OrbiSIMS**, ***Anna Kotowska,*** *M. Edney,* University of Nottingham, UK*; A. Shard,* National Physical Laboratory, UK*; J. Aylott, M. Alexander, D. Scurr,* University of Nottingham, UK

In contrast to biological imaging methods such as fluorescence microscopy, SIMS has the capability to map several groups of compounds simultaneously in an untargeted way, without labelling. Particularly, reduced fragmentation offered by the GCIB primary beam and high mass resolving power of the Orbitrap™ analyser enable detailed characterisation of biological samples[1]. Lipids, metabolites and proteins, which previously would have been undistinguishable in complex samples due to the limited mass resolving power of ToF-SIMS, can be assigned in the spectra. However, this advantage of the 3D OrbiSIMS cannot be fully utilised because of large volume (tens of thousands of peaks) and complexity (diverse chemistry) of real biological samples.

Here, we have developed a chemical filtering process by the application of molecular formula prediction (MFP) and the level of molecule saturation (double bond equivalent) to separate multidimensional SIMS data[2]. This approach is particularly advantageous in 3D OrbiSIMS data, which contains mixtures of molecular ions as well as several fragment ions per molecule. Furthermore, we integrated the LIPIDMAPS® database and generated a protein fragment database to facilitate chemical filtering and assignment of these molecules. Our chemical filtering method has been successfully applied to challenging biological samples, assigning salts, lipids and protein fragments in human serum[2], mapping different lipid classes throughout human skin[3] and tracking lipids, polysaccharides, glycolipids and protein fragments in a bacterial biofilm[4].

This work describes the interpretation of complex biological datasets using the chemical filtering approach. Particularly, it focuses on the chemical filtering and assignment of poorly ionisable molecules (e.g. protein fragments), which are likely to be missed in statistical analysis. In addition to filtering protein fragments, this method enables rapid assignment and classification of protein ions. This approach can enable progress in predicting which fragments will be seen in the 3D OrbiSIMS spectrum and identify proteins in an analogous way as the proteomics community has developed for liquid chromatography MS.

1. Passarelli, M.K. *et al.* The 3D OrbiSIMS - Label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power. *Nat. Methods,* 2017
2. Edney, M.K. *et al.* Molecular Formula Prediction for Chemical Filtering of 3D OrbiSIMS Datasets. *Anal. Chem.*, 2022
3. Starr N.J. *et al.* Elucidating the molecular landscape of the stratum corneum. *PNAS*, 2022
4. Kotowska A.M. *et al.* under review 2022

9:40am **DI-ThM1-7 Time-of-Flight Sims Investigation of Isobaric Oligopeptides**, ***Alessandro Auditore,*** Università di Catania, Italy*; N. Grasso,* Università di Catania*; N. Tuccitto, A. Licciardello,* Università di Catania, Italy

Mass Spectrometry methods are widely used analytical techniques for structural characterization of biological molecules. Soft ionization techniques such as ESI and MALDI allow easy determination of the molecular mass and, through the fragmentation patterns, enable the sequencing of linear biomolecules. In this framework, the capability to distinguish between isobaric species has always been a challenging analytical task[1], which is normally tackled by complex mass spectrometric approaches[2]. Often, this holds even in the case of relatively low mass systems such as oligopeptides. On the other hand, time-of-flight secondary ion mass spectrometry, due to the peculiar emission/ion formation mechanisms, often does not allow the detection of the molecular ion of proteins and peptides, while it gives rise to a rich fragmentation pattern which can be used for recognizing, for example, surface adsorbed proteins, often with the help of multivariate analysis methods[3]. Indeed, the different aminoacidic sequence in the oligopeptide chains is expected to determine a different fragmentation pattern in the spectrum. Due to the relevant number of peaks in the SIMS spectra of peptides and protein and the slight differences in intensities between different samples, the multivariate analysis approach allows an easier interpretation of the information included in the ToF-SIMS spectra.

In the present work we investigated four peptides with high similarity in the aminoacid sequence along the peptide chain. The reference peptide (TAT1) is a 12-unit sequence of six aminoacids (GRKKRRQRRRPS). The other

three peptides have been obtained by inserting a bAla-H dipeptide (carnosine) in three different positions inside the TAT1 chain, leading to three isobaric molecules, namely GRKKRRQRRRPS-bAla-H (TAT1-Car), bAla-HGRKKRRQRRRPS (Car-TAT1) and GRKKRRQ-bAla-H-RRRPS (T-Car-T), not easily distinguishable each other even by means of conventional MS-MS techniques. We show that these oligopeptides can be easily distinguished by ToFSIMS if deposited onto a surface and after multivariate data analysis of the spectra. Additional information will be provided on the results obtained by deposition on different types of surfaces.

[1]Y. Zhang et al. *J. Am. Soc. Mass Spectrom.* 16 (2005) 1827–1839.

[2]N. Pappireddi et al. *J. Chem. Biol*. 20 (2019) 1210–1224.

[3]S. Muramoto et al, The J. Phys. Chem. C, 115 (2011) 24247-24255.

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