

SIMS Solutions in Materials and Life Sciences Room Great Lakes B - Session SS+DI-MoA3

Industrial Applications II

Moderators: Cody Cushman, Corning Incorporated, Teruaki Kikuchi, SONY Semiconductor Manufacturing

2:00pm **SS+DI-MoA3-1 Keynote Industrial Talk: Correlative Microscopy and Data Analysis for Semiconductor Technology Applications**, *Jean-Paul Barnes*, C. Guyot, P. Hirchenhahn, N. Gauthier, M. Moreno, T. Maindron, Y. Mazel, E. Nolot, CEA-Leti, France; A. Priebe, EMPA (Swiss Federal Laboratories for Materials Science and Technology), Switzerland; B. Gautier, CNRS, France; A. Tempez, S. Legendre, HORIBA France; G. Fisher, Physical Electronics USA

INVITED

The increasingly complex structures and large variety of materials used in modern nano and opto-electronic devices drives the need to develop new approaches for their characterization. To obtain the desired information it is often necessary to combine several techniques to acquire reliable information. Ideally, this should be from exactly the same spot on a specimen. This can be challenging both in terms of measurement protocols, but also in the data treatment required to correlate data sets from different techniques and/or modalities. In an applied research or industrial environment, the ability to give fast feedback is a great advantage in materials and process development. It is thus important to have access to a wide range instruments and techniques that are complementary in their capabilities. This presentation will address developments in TOF-SIMS and tandem MS analysis for applications from semiconductor technology to display technology and the importance of using several techniques such as scanning probe microscopy, X-ray tomography, TEM-EDX, XPS and plasma profiling time-of-flight mass spectrometry. The importance of sample preparation to enable multi-technique studies is also critical and several examples will be given involving focused ion beam milling, wedge crater preparation and transfer between instruments under a protected environment (vacuum or inert gas).

Part of this work, carried out on the Platform for Nanocharacterisation (PFNC), was supported by the "Recherches Technologiques de Base" program of the French National Research Agency (ANR).

2:40pm **SS+DI-MoA3-5 Basic Evaluation and Impurity Analysis in OLED Devices with New Ion Guns for Dynamic-SIMS**, *Tomomi Ohashi*, S. Inayoshi, ULVAC, Inc., Japan; D. Sakai, T. Miyayama, ULVAC PHI, Inc., Japan ULVAC-PHI developed new ion guns that can narrow the diameter of the beam. These have been installed in our own D-SIMS equipment (ADEPT-1010). We report that the results from the basic evaluations and impurity analyses of OLED devices.

We analyzed small areas (100 μm x 250 μm) of patterned samples. Samples were Au/Pt/Ti/Si wafers as multilayer films. These were compared before and after annealing (Fig. 1①). It was observed that Au diffused toward the Pt film in the sample after annealing. The results of measuring larger areas (Fig.1②) were equivalent to the results of measuring the smaller areas. Therefore, it has been determined that measuring smaller areas were possible without being affected by the surrounding area.

It is known that the OLED device lifetime is shortened if impurities are mixed in during their production. We evaluated the intensity and the in-plane distribution of impurities in OLED devices, especially focusing on halogen elements in the organic layers. We prepared two samples. One is the sample **1 with a short lifetime and the other is the sample **1 with a long lifetime. The device has a light-emitting area of 2.3 mm in diameter. The luminous area was divided into five regions (top, bottom, left, right, and center), and each location was measured multiple times. Fig. 2 shows the results at the center of the sample. Fluorine was detected from devices with shorter lifetimes, but there was no difference in chlorine intensity. Similar results were obtained from other regions. To evaluate reproducibility, we compared the Coefficient of Variation (CV) of the integrated intensity values of fluorine ($m/z = 19$) from 250 s to 450 s. The CV of the center position was 0.090, and the overall CV ranged from 0.050 to 0.17. Although there was some variation, the reproducibility was generally good. These results suggest that one of the reasons for the shorter lifetime of OLED devices is the presence of fluorine in the organic layer.

References:[1] K. Suzuki et al. Proc. of the 33th Meeting of Japan OLED Forum, (2021), p. 13.

3:00pm **SS+DI-MoA3-7 Sample Processing by Bi-FIB for TOF-SIMS Imaging of Buried Interfaces**, *Shin-ichi Iida*, ULVAC-PHI, Inc., Japan; G. Fisher, Physical Electronics; T. Miyayama, ULVAC-PHI, Inc., Japan

Focused ion beam (FIB) is commonly used as a standard machining technique in failure analysis, quality control, reverse engineering, material research, etc., for the samples having micro- and nanostructures. FIB combined with time-of-flight secondary ion mass spectrometry (TOF-SIMS), so-called FIB-TOF, has attracted attention as a method to determine the three-dimensional (3D) chemical distributions of complex samples. In general, a highly focused Ga⁺ ion beam is used for FIB, however, the FIB-milled area is limited and it was difficult to expand the Ga-FIB to hundreds of micron length scale sample fabrication. In order to overcome the drawback, we proposed Bi-FIB approach for large scale sample cross-sectioning. Although the possibility of Bi-FIB has been reported, there were almost no performance examinations as well as practical applications. In this study, therefore, the authors summarize the comparison of milling rate and milling damage between Ga-FIB and Bi-FIB. As a result, it was found that Bi-FIB can provide higher milling rate with thinner milling damage. Finally, the Bi-FIB approach was applied to the interfacial analysis of all-solid-state battery (ASSB) material, because the functionality of ASSBs strongly depends on the solid/solid interface. With this approach, the detailed chemical distributions at the interface was discovered, leading to the better understanding of battery behaviors.

3:20pm **SS+DI-MoA3-9 HDR of SIMS Data**, *Henrik Arlinghaus*, D. Rading, E. Niehuis, IONTOF GmbH, Germany

The number of secondary ions generated during a ToF-SIMS experiment is dependent on numerous factors. While the operator is able to configure the instrument to optimize the yield, many factors such as the ionization probability, differences in molecular species concentrations, or the (in)homogeneity of the spatial distribution within the sample cannot be changed. Challenging samples may therefore run into the limits of the dynamic range of a modern SIMS instrument, which is around five orders of magnitude. When this is the case the operator must find a compromise which limits the noise in low intensity signals and areas while minimizing oversaturation of high intensity signals or areas.

In photography one approach to overcome similar limitations is the use of "High Dynamic Range", or HDR photography. This approach takes multiple images in short succession with varying exposure times and then fuses these together to generate a single composite image.

Previously we had demonstrated the possibility of acquiring multiple datasets during a single acquisition pseudo simultaneously, with each dataset having been acquired using different instrument parameters, while minimizing the impact of changes in the sample or the environment, using multiplexing[1]. In this paper, we continue that work by demonstrating that it is possible to generate combined profile and images for substances of interest using a HDR-like algorithm from such a dataset, reducing noise within low intensity areas, and saturation effects in high intensity areas, simplifying data interpretation.

[1]: Multiplexing ToF-SIMS acquisition modes to improve information yield

Dealing with Data and Interpretation Room Great Lakes Promenade & A1 - Session DI-TuP

Dealing with Data and Interpretation Poster Session

DI-TuP-1 Statistical Analysis of ToF-Sims Images: Seeking Patterns in the Noise, *Alan Spool*, Western Digital Corporation

TOF-SIMS analysts are often asked to discern difficult to find features such as evidence for corrosion, small particles, features at the edge of our detection limits, and other hints of inhomogeneity. The human ability to recognize patterns can also lead to the discernment of non-existent features, or at least uncertainty about whether an image is truly random. A simple statistical test, comparing a calculated Poisson distribution to the actual distribution accompanied by a chi square test solves this problem. If the distributions match, the image variations are random noise.

Data that is non-random should be over dispersed. Variability within the image should produce a wider range of pixel counts, more pixels with higher counts than expected, and more with less. However, this work shows that TOF-SIMS images are often under dispersed, that is, a narrower distribution than calculated. This tends to be more of an issue with ions showing higher intensities. Dead time in the instrument tends to artificially reduce the signal overall, and it reduces the probability of pixels having higher counts more than it reduces the probability of them having lower counts, thus narrowing the distribution. It is still possible to apply the test by adjusting the data. For a Poisson distribution, the variance is equal to the mean. One can adjust the data by subtracting the difference between the variance and the mean from all of the pixel count values, thus creating a distribution where, given no other reasons for variations, the variance equals the mean.

In this work, various data sets are tested by this method and the results discussed. The use of this method turns out to be equally useful to the evaluation of image data and the evaluation of instrument artifacts.

DI-TuP-3 Characterisation of Noise in the Orbisims and Scaling Method for More Effective Multivariate Data Analysis, *Michael R. Keenan*, Independent; *G. Trindade*, National Physical Laboratory, UK; *A. Pirk*, IONTOF GmbH, Germany; *J. Zhang*, National Physical Laboratory, UK; *H. Arlinghaus*, IONTOF GmbH, Germany; *L. Matjacic*, National Physical Laboratory, UK; *C. Newell*, The Francis Crick Institute, UK; *R. Havelund*, National Physical Laboratory, UK; *K. Ayzikov*, Thermo Fisher Scientific, Germany; *A. Gould*, The Francis Crick Institute, UK; *J. Bunch*, National Physical Laboratory, UK; *A. Makarov*, Thermo Fisher Scientific, Germany; *I. Gilmore*, National Physical Laboratory, UK

The most challenging measurements are often at the boundary of detection just above the noise, for example the detection of gravitational waves where an understanding of the detector noise was critical.¹ A study of the noise in a detector system is of wider importance and a better understanding can make a profound difference to measurement sensitivity, reproducibility, and the interpretation. It can have an important contribution to the variance in data that may even overbear biological sample-to-sample variance and is essential for correct use of multivariate based data analytics.

The OrbisIMS instrument² features a Time-of-Flight (ToF) mass spectrometer (MS) and an Orbitrap MS, which confer advantages of speed and high mass resolution, respectively. Secondary ions are accelerated by an extraction electrode and can either pass directly through a switching electrode to the ToF MS or can be deflected to a transfer system that sends them towards the Orbitrap MS. The ToF MS uses a channel plate detector in a single ion counting mode and Poisson-distributed secondary ions are convolved with detector deadtime effects to yield binomially-distributed signal.³ In contrast, the Orbitrap analyser uses a quasi-continuous source of secondary ions that are injected into an ion trap where they revolve around the central electrode and oscillate along spindle shaped electrodes with a frequency inversely proportional to the square root of the mass of the ion. An image charge is created in the pair of outer electrodes and is measured with time. This time-domain transient signal is converted to frequency (and hence mass) domain by a Fourier transform.

Measurement of the noise distribution of an Orbitrap analyser requires a stable source of ions. Here, we take advantage of a Bi-Nanoprobe (IONTOF GmbH) that has a very stable (< 1% RSD) 30 keV Bi₃⁺ primary ion beam. We report measurements across a range of ion intensities and developed a statistical model that considers three sources of noise: "counting noise";

"transfer noise" of ions into the Orbitrap analyser; and "thermal noise" from the Orbitrap detection circuit. This model was used to develop a data scaling strategy that accounts for heteroscedasticity (non-uniform noise). We show that our scaling strategy has important implications for Principal Component Analysis (PCA), similarly to what has been developed before for the noise in ToF-SIMS.³

1. B. P. Abbott *et al.* *Phys. Rev. Lett.*, 116, 1–16, 2016
2. M. K. Passarelli *et al.* *Nat. Methods*, 14 1175–1183 2017
3. M. R. Keenan and V. S. Smentkowski, *Surf. Interface Anal.*, 48 218–225, 2016

DI-TuP-5 4D Surface Reconstruction to Link Microstructural Topography with Sims Information, *Jean-Nicolas Audinot*, A. Ost, Luxembourg Institute of Science and Technology (LIST), Luxembourg; *T. Wirtz*, Luxembourg Institute of Science and Technology (LIST), Luxembourg

Surface topography is known to have a strong influence on secondary ion emission under primary ion bombardment. Topography variations induce local changes of the incidence angle of the primary ion beam, strongly affecting surface sputtering processes, and hence the sputtering yield [1]. SIMS images suffer from topographical artefacts, resulting from these local variations of the sputtering yield, which can lead to erroneous conclusions about materials' surface concentration gradients.

In the recent years, we have developed and improved a method for 3D reconstruction of samples with complex surfaces from multi-view Secondary Electron (SE) images correlated with analytical SIMS images [2,3]. A series of SE images is taken at different angles around the sample and implemented into a photogrammetry software allowing to obtain a 3D SE surface model. Subsequently, the SIMS image is acquired in top view mode and projected onto the 3D SE reconstruction to obtain a full 4D surface model. Using a numerical processing algorithm, topographical information is extracted from the reconstruction and linked to the local intensity of the SIMS signal to better understand intrinsic properties of the material.

In this contribution, we will review the 4D methodology by showing applications from different fields (materials science and geology). The data was obtained both on commercial instruments (SIMS data from a CAMECA NanoSIMS 50L correlated with SE data obtained on a Secondary Electron Microscope) and on in-house developed instruments (SIMS and SE data from a Helium Ion Microscope equipped with a magnetic sector SIMS add-on system) [4]. 4D results are useful not only for enhanced specimen visualization, but also to study variations of the local topography to learn more about nano-scale material transformation processes and to localize and correct SIMS image artefacts.

- [1] H. L. Bay & J. Bohdanský, *App. physics* 19, (1979), p. 421
- [2] F. Vollnhals, T. Wirtz, *Anal. Chem.* 90 (2018), p. 11989
- [3] A.D. Ost *et al.*, *Environ. Sci. Technol.* 55 (2021), p. 9384
- [4] T. Wirtz *et al.*, *Annu. Rev. Anal. Chem.* 12 (2019), p. 523

DI-TuP-7 Comparison Study of Mouse Brain Tissue by Using ToF-SIMS with Static Limit and Hybrid SIMS Beyond Static Limit (Dynamic Mode), *Hyun Kyong Shon*, *J. Son*, Korea Research Institute of Standards and Science (KRISS), Republic of Korea; *J. Moon*, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Republic of Korea; *J. Jim*, Korea Basic Science Institute (KBSI), Republic of Korea; *T. Lee*, Korea Research Institute of Standards and Science (KRISS), Republic of Korea

The mouse brain is widely used in various studies, including studies on degenerative brain diseases such as Alzheimer's and Parkinson disease [1–3]. In particular, several attempts have made to measure disease-related biomolecules such as metabolite, fatty acid, and lipids from tissue images of mouse brain by using cluster ion beam in time-of-flight secondary ion mass spectrometry (ToF-SIMS). To know exactly what biomolecules in the ToF-SIMS images are, ToF-SIMS equipments with MS/MS function to identify biomolecules have recently been released [4–6].

In this study, the mouse brain was sectioned approximately bregma +1 mm point in the coronal section, and mass spectra and images were obtained by using argon cluster ion beam. It is intended to compare the mass spectra from TOF-SIMS within static limit with those from Hybrid SIMS beyond static limit, i.e., dynamic mode. In particular, the outer-cortex layer, corpus callosum, caudate-putamen, and piriform region are compared in detail.

- [1] W. Michno, P. M. Wehrli, K. Blennow, H. Zetterberg, J. Hanrieder, *Journal of Neurochemistry*, 151, 2019, 488–506.

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[2] L. Carlred, V. Vuckjević, B. Johansson, M. Schalling, P. Sjövall, *Biointerphases*, **11**, 2016, 02A312

[3] S. Solé-Domènech, P. Sjövall, V. Vukojević, R. Fernando, A. Codita, S. Salve, N. Bogdanović, A. H. Mohammed, P. Hammarström, K. P. R. Nilsson, F. M. LaFerla, S. Jacob, P. Berggren, L. Giménez-Llor, M. Schalling, L. Erenius, B. Johansson, *Acta Neuropathol*, **125**, 2013, 145-257.

[4] P. Agüi-Gonzalez, S. Jähne, N. T. N. Phan, *Journal of Analytical Atomic Spectrometry*, **34**, 2019, 1355-1368.

[5] M. K. Passarelli, A. Pirk, R. Mollers, D. Grinfeld, F. Kollmer, R. Havelund, C. F. Mewman, P. S. Marshall, Henrik Arlinghaus, M. R. Alexander, A. West, S. Horning, E. Niehuis, A. Kakarov, C. T. Dollery, I. S. Gilmore, *Nature Methods*, **14**(12), 2017, 1175-1183.

[6] A. M. Kotowska, G. F. Trindade, P. M. Mendes, P. M. Williams, J. W. Aylott, A. G. Shard, M. R. Alexander, D. J. Scurr. *Nature Communications*, **11**, 2020, 5832

networks (ANNs) for polymer analysis or protein analysis.[2,3] Here, we classified similar type of microplastic products using the self-organizing map (SOM) method, which is a type of ANNs. Through this, we were able to successfully distinguish 5 types of plastics that could not be distinguished by PCA.

[1] H. Mei, *et al*, *J. Polym. Sci.* **2022**, *60*, 1174-1198

[2] R. M. T. Madiona, *et al*, *Anal. Chem.* **2018**, *90*, 12475-12484

[3] N. G. Welch, *et. al*. *Langmuir*, **2016**, *32*, 8717-8728

DI-TuP-9 Depth Correction of 3D NanoSIMS Images Show Intracellular Lipid and Cholesterol Distributions While Capturing the Effects of Differential Sputter Rate, Melanie Brunet, B. Gorman, M. Kraft, University of Illinois Urbana-Champaign

Changes in the distributions of cholesterol and various lipid species within cells are correlated with diseases such as Niemann-Pick, influenza, SARS CoV-2, and HIV. Visualization of the spatial distribution of lipids and other biomolecules could provide new insight into their roles in cellular function and disease. Our lab has used NanoSIMS in a depth profiling mode to image metabolically incorporated, rare stable isotope-labeled cholesterol and sphingolipids within mammalian cells. This depth profiling produced a series of 2D NanoSIMS images depicting the same location on the cell but at progressively increasing depth from its surface. When SIMS depth profiling images of nonplanar samples (e.g., cells) are sequentially stacked to form a 3D image, the component-specific secondary ions detected in the same scan are positioned at the same height in the 3D image. In contrast, the molecules that produced these ions were located at different heights above the substrate. This discrepancy distorts the 3D image in the z-direction. Although 3D SIMS images may be depth corrected with strategies that require atomic force microscopy (AFM) data or the detection of additional secondary ions from the substrate, approaches for depth correction in the absence of such complementary data are desired. Thus, we developed a method to depth correct 3D NanoSIMS depth profiling images of cells that accounts for the effects of differential sputter rate. Our method reconstructs the cell's morphology at each raster plane using the secondary ion and secondary electron depth profiling images. These morphologies are used to adjust the z-positions and heights of the voxels in the component-specific 3D NanoSIMS images. To validate our method, we reconstructed cell morphologies from depth profiling images collected using focused ion beam – secondary electron microscopy (FIB-SEM) and compared them to correlated AFM data. The shape of the reconstructed morphologies agreed well with the AFM data, with an average accuracy of 90%. Intracellular features containing sphingolipids or cholesterol were better resolved in depth corrected 3D NanoSIMS images. Because this method uses only the secondary electron and secondary ion images generated during negative ion SIMS depth profiling, depth corrected 3D images for existing depth profiling SIMS datasets may now be created in the absence of correlated topography data. Application of this method to depth profiling SIMS data of cells may shed light on the mechanisms behind changes in the distributions of cholesterol and various lipid species in disease and facilitate the identification of organelles enriched with biomolecules of interest.

DI-TuP-11 Microplastic Products Discrimination with ToF-Sims Using the Clustering Self-Organizing Maps (SOM), Jin Gyeong Son, H. Shon, Korea Research Institute of Standards and Science (KRISS), Republic of Korea; J. Kim, Airiss, Republic of Korea; T. Lee, Korea Research Institute of Standards and Science (KRISS), Republic of Korea

ToF-SIMS is a surface chemical analysis instrument that provides information at the molecular level of the surface. It has been utilized in the field of polymers to analyze composition using backbone-specific repeating units and to distinguish copolymer components.[1] However, due to the intricacy of ToF-SIMS data, it is still challenging to differentiate chemically similar types of polymers.

Principal component analysis (PCA), which has been widely used in ToF-SIMS analysis, is difficult to distinguish chemically similar data due to the manual assignment of peaks and relatively simple linear clustering or dimensionality reduction methods. Recently, there is a report that large-scale multivariate data can be classified and clustered using artificial neural

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. IMSF 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 task¹, which is normally tackled by complex mass spectrometric approaches². 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 methods³. 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 (GRKKRRRRPS). The other

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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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— K —

Keenan, M.: DI-TuP-3, **2**
Kim, J.: DI-TuP-11, 3
Kotowska, A.: DI-ThM1-5, **4**
Kraft, M.: DI-TuP-9, 3

— L —

Lee, T.: DI-TuP-11, 3; DI-TuP-7, 2
Legendre, S.: SS+DI-MoA3-1, 1
Licciardello, A.: DI-ThM1-7, 4

— M —

Maindron, T.: SS+DI-MoA3-1, 1
Makarov, A.: DI-TuP-3, 2
Matjacic, L.: DI-TuP-3, 2
Mazel, Y.: SS+DI-MoA3-1, 1
McHardy, K.: DI-ThM1-3, 4
Miyayama, T.: SS+DI-MoA3-5, 1; SS+DI-MoA3-7, 1

Moon, J.: DI-TuP-7, 2

Moreno, M.: SS+DI-MoA3-1, 1

— N —

Newell, C.: DI-TuP-3, 2

Niehuis, E.: SS+DI-MoA3-9, 1

Nolot, E.: SS+DI-MoA3-1, 1

— O —

Ohashi, T.: SS+DI-MoA3-5, **1**

Ost, A.: DI-TuP-5, 2

— P —

Pirkel, A.: DI-TuP-3, 2

Priebe, A.: SS+DI-MoA3-1, 1

— R —

Rading, D.: SS+DI-MoA3-9, 1

Ryszka, M.: DI-ThM1-3, **4**

— S —

Sakai, D.: SS+DI-MoA3-5, 1

Sano, N.: DI-ThM1-3, 4

Scurr, D.: DI-ThM1-5, 4

Shard, A.: DI-ThM1-5, 4

Shon, H.: DI-TuP-11, 3; DI-TuP-7, **2**

Son, J.: DI-TuP-11, **3**; DI-TuP-7, 2

Spool, A.: DI-TuP-1, **2**

Stickland, A.: DI-ThM1-3, 4

— T —

Tempez, A.: SS+DI-MoA3-1, 1

Trindade, G.: DI-TuP-3, 2

Tuccitto, N.: DI-ThM1-7, 4

Tyler, B.: DI-ThM1-1, **4**

— W —

Wirtz, T.: DI-TuP-5, 2

— Z —

Zhang, J.: DI-TuP-3, 2