Tuesday Evening, September 20, 2022

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. Pirkl,* 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 instrument[2] 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 Bi_3^+ 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]

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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.

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[2] F. Vollnhals, T. Wirtz, Anal. Chem. 90 (2018), p. 11989

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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 Instutue 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 Sicence Institute(KBSI), Republic of Korea*; T. Lee,* Korea Research Instutue 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.

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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)

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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 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.

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