Applied Surface Science
Room: 316 - Session AS+BI+MC-WeM

Chemical Imaging in 2D and 3D

Moderator: Jeffrey Fenton, Medtronic, Inc., Kathryn Lloyd, DuPont Corporate Center for Analytical Sciences

8:20am **AS+BI+MC-WeM2 Expanded Approaches for Single Cell Analysis by SIMS**, ***Christopher Szakal***, National Institute of Standards and Technology (NIST)

Secondary ion mass spectrometry (SIMS) has been increasingly utilized for single cell imaging owing to its unique combination of spatial resolution and chemical differentiation by mass. Depending on the instrument type, subcellular lateral resolution between 10's and 100's of nanometers can be obtained, sometimes with both elemental and organic information obtained simultaneously, and sometimes with highly precise isotopic ratio measurements being attainable. However, imaging at the limits of the technique requires sufficient counts per pixel, which can be limited by analyte concentrations, competitive ionization pathways, and cumulative cluster ion beam damage accumulation. This work focuses on the advantages and disadvantages of combining focused ion beam (FIB) milling of single cells with subsequent ToF-SIMS imaging, as well as using large geometry (LG)-SIMS for high mass resolution analysis of single cell components that would otherwise not be easily detectable in other instrumental configurations. Such developments expand the research areas that are possible for single cell SIMS analyses, including cell differentiation without relying on multivariate analyses and targeted cell uptake studies.

8:40am **AS+BI+MC-WeM3 3-Dimensional Chemical Imaging on the Nanoscale with Cluster-SIMS**, ***Nicholas Winograd***, Penn State University **INVITED**

Bombardment of molecular solids with polyatomic projectiles allows interrogation of the sample with reduced chemical damage accumulation. Hence, it is now possible to perform depth profiling experiments with a depth resolution of less than 10 nm. In our hands, the projectile of choice is C_{60} due to the fact that the ion beam can be focused to a 250 nm spot size, and erosion of the sample can be performed with minimal chemical damage, especially at low temperature. With this combination of properties, it is feasible to think about creating 3-dimensional molecule-specific images.

A basic impediment to accomplishing this goal involves the fact that the SIMS images provide only chemical information and no direct depth information. The measureable quantity is the incident ion beam fluence, which can indirectly be related to depth, but independent measurements are required. The formation of topography and differential sputtering effects across the sample surface can also degrade the quality of the 3-D rendering when 2-D images are stacked. We have employed AFM in combination with SIMS imaging to develop protocols for correcting for these phenomena. Here, examples are shown using a patterned trehalose thin film and an Irganox delta layer reference material provided by NPL in the U.K. The idea is to provide chemical information with SIMS, and the depth information, acquired at each pixel in the image, using AFM. In addition to examining eroded craters directly, we have also developed a wedge-beveling technique that allows sputtering yield and topography to be determined with a single SIMS measurement and a single AFM measurement.

The long term aim of developing these protocols is to be able to acquire high resolution chemical images of single biological cells. So far, it appears that differential sputtering effects are not too serious for these samples. The combined SIMS/AFM strategy developed here will be important for verifying these initial observations. Finally, there is an emerging interest in gas cluster ion sources, namely Ar_{4000}, since even less chemical damage than C_{60} is observed, and the depth resolution during erosion appears to be less than 5 nm. Here we show that the combination of C_{60} imaging and Ar_{4000} sputtering provides an even more powerful protocol. In general, we show that the AFM/SIMS combination is a powerful tool for 3-dimensional chemical imaging.

9:20am **AS+BI+MC-WeM5 SIMS 2D and 3D Characterization of Organic/Inorganic Surfaces by FIB Crater Wall Imaging and Tomography**, ***Felix Kollmer***, *R. Möllers, D. Rading, S. Kayser,* ION-TOF GmbH, Germany, *N. Havercroft,* ION-TOF USA, Inc., *E. Niehuis,* ION-TOF GmbH, Germany

Information on the chemical composition, physical properties and the three dimensional structure of materials and devices is of major importance. Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) is known to be an extremely sensitive surface imaging technique which provides elemental as well as comprehensive molecular information on all types of solid surfaces. In the so-called dual beam mode the pulsed analysis beam is combined with a low energy sputter ion beam for the removal of material. This allows depth profiling of multilayers with high depth resolution as well as three-dimensional analysis.

However, the analysis of structures at greater depth (> 10µm) requires long measurement times and the build-up of surface roughness at the crater bottom limits the achievable spatial resolution. Moreover, extremely rough samples, samples with voids, and material that exhibits strong local variations in density or sputter yield are unsuitable for conventional depth profiling. Not only that the initial surface topography is unknown but it is also modified and in many cases even roughened by the sputtering process.

In order to overcome these limitations we used a combined SIMS/FIB setup. Either a Bi cluster beam or a mono-atomic Ga beam is used to FIB mill a crater into the sample. Subsequently, a 2D TOF-SIMS image of the vertical crater wall is acquired. Since the crater wall is hardly affected by the aforementioned roughening problems this approach allows the in-depth distribution of elements to be determined by analyzing a plane perpendicular to the surface at high lateral resolution (Dl<50nm) [1].

Moreover, by serial slicing of the crater wall followed by intermediate analysis steps this approach can be extended in order to provide the full 3D characterization of the analyzed volume. We will present 2D and 3D data of reference material, multilayer samples and technically relevant real world samples such as fuel cells and battery electrodes. For thin multilayer samples the FIB process can be performed under grazing incidence in order to bevel the surface and hence magnify and accentuate thin layers in the plane of the analyzed crater wall.

However, the FIB/SIMS approach fails when analyzing organic surfaces since the molecular structure is almost completely destroyed by the sputtering process. We will discuss methods to maintain the molecular structure under high dose sputtering conditions by performing the FIB milling with massive argon clusters.

[1] F. Kollmer, W. Paul, M. Krehl, E. Niehuis, SIMS XVIII proceedings paper, Surf. Interface Anal., 2012

9:40am **AS+BI+MC-WeM6 Multivariate Imaging: A New Approach towards Chemical State Identification of Novel Carbons in XPS Imaging**, ***Anders Barlow***, *N. Sano, P.J. Cumpson*, NEXUS, Newcastle University, UK

The differentiation between various forms of carbon in XPS spectra is made difficult by the subtle changes in C1s spectra that one would typically analyse. This is ideally demonstrated by a comparison of sp^2 and sp^3 carbon, such as graphite and diamond, where the variation in the C1s peak is less than 1eV. When applied to 'real' samples, such as a diamond like carbon coating, or a graphene surface, this difference can be even less. This presents a real problem for XPS imaging, where typically the analyst would sacrifice energy resolution in favour of signal intensity and spatial resolution. Such subtle differences are then completely lost when performing XPS imaging of novel carbon surfaces, where there may be discrete boundaries or layers between materials that are chemically very different, yet appear the same when the C1s peak energy is used in imaging.

We report a method of elucidating these differences in XPS imaging through shifting the focus from the C1s feature, to the X-ray induced Auger feature, a method we call Multivariate Auger Feature Imaging (MAFI). The carbon Auger feature can be studied and through the extraction of the so-called D-Parameter[1], chemical states of carbon can be clearly identified, with little ambiguity between sp^2 and sp^3 states. Extension of this method to XPS imaging, and the generation of 3-Dimensional images (2 spatial, 1 kinetic energy), we have shown that imaging of the Auger feature of graphite on polymers can identify multiple states of carbon-carbon bonding domains, where the imaging of the C1s feature alone yields no distinguishable differences or spatial features. We have also shown that PCA analysis of the carbon Auger feature also yields clear and distinguishable differences in the XPS images. The result is two independent methods of distinguishing novel carbon materials from one-another in XPS imaging. With modern instrumentation capable of a spatial

resolution down to the few micron level, this greatly enhances the capability of XPS instrumentation to image novel carbon surfaces and devices.

[1] Lascovich, J.C. et al., App. Surf. Sci., 47(1), pp. 17-21 (1991).

11:00am **AS+BI+MC-WeM10 Multivariate Analysis Approaches for Image De-noising and Image Fusion**, ***Bonnie Tyler***, National Physical Laboratory (NPL), UK **INVITED**

Image fusion has become widely used in both medical diagnostics and optical remote sensing and there is growing interest in using these methods in applied surface science research. The goal of data fusion is to combine measurements from complementary techniques in order to aid in the analysis of the data and enhance information content. Recently, pan-sharpening techniques developed for optical remote sensing have received considerable interest in the surface science community because of their ability to improve spatial resolution and image contrast. Although image fusion can produce dramatic improvements in image sharpness and contrast, it can also lead to significant artefacts and care must be taken to ensure reliable results. These artefacts can be quite severe if the spectra have sharp bands, high background, or low signal-to-noise, features that are common in ToF-SIMS and XPS imaging. For optical remote sensing, a wide variety of methods have been developed for pan-sharpening, including approaches based on wavelet transforms, high pass filters, intensity hue saturation, Gram-Schmidt transforms, and Principal Components Analysis. Each of these methods offers advantages for certain applications but all are prone to artefacts when applied under non-optimal conditions. In order to minimize artefacts and produce reliable results, the methods must be adapted to account for the unique characteristics of different imaging modes. Of the methods in the literature, PCA image fusion is the most readily adapted for use with ToF-SIMS and XPS images. Methods for adapting PCA fusion for optimal use with ToF-SIMS and XPS images will be presented, including statistically based preprocessing of the data, target factor rotations and histogram matching. PCA image fusion can be a valuable technique for reducing noise, improving image contrast, and spatial resolution in ToF-SIMS and XPS data. With appropriate attention to the unique characteristics of each spectrometry, this can be done without significant artefacts or distortion of the spectral detail.

11:40am **AS+BI+MC-WeM12 Global Analysis Peak Fitting for Imaging NEXAFS Data**, ***Mark H. Van Benthem***, *J.A. Ohlhausen*, Sandia National Laboratory

We will present a method of analyzing NEXAFS image data to extract chemical information from the complex elemental peak structure in the material under analysis. The method, known as global analysis, fits emission bands to peaks described by nonlinear functions using nonlinear and linear optimization techniques. It can fit multiple types of peaks simultaneously, such as those found in NEXAFS spectra: Gaussian, Lorentzian, Voigt, asymmetric Gaussian and Lorentzian, and step edge with decay. Typically, peak fitting of NEXAFS data is very complex and somewhat arbitrary. Our method takes advantage of the high dimensionality of the image space to yield peaks with potentially greater reliability than single spectrum fitting. The method also employs data compression with principal component analysis (PCA) to rapidly complete the analysis. A discussion of the algorithm along with several examples of its application will be presented.

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12:00pm **AS+BI+MC-WeM13 Visualizing Pharmaceutical Compounds in Single-cells with label-free 3D Mass Spectrometry Imaging**, ***Melissa K. Passarelli***, *C. Newman*, National Physical Laboratory, UK, *A. West*, University of York, UK, *C.T. Dollery*, *I.S. Gilmore*, National Physical Laboratory, UK, *J. Bunch*, National Physical Laboratory

Drug-induced phospholipidosis is an adverse side-effect that hinders the therapeutic value of some pharmaceutical compounds. In this report, three-dimensional secondary ion mass spectrometry (SIMS) imaging was used to investigate the cellular uptake of phospholipidosis–inducing pharmaceutical compounds. A fast and simple sample preparation method, frozen dehydrated, was used to extract the drug compound to the surface layers of individual cells. Although the native localization of drug compound within the cell is lost, the compound was isolated to the confines of the individual cells and matrix-related effects were no longer a concern. With this method we were able to successfully detect intact-unlabeled drug compound at therapeutic dosages in macrophages. Relative quantification of the drug compound in individual cells was achieved. Overall, this approach provides a platform for studying cellular uptake of pharmaceutical compounds at the single cell level. This system also provides a model for studying metrology of cell imaging using SIMS. The effects of sample preparation and limitations of current technologies will be discussed along with new possibilities for the future.

Authors Index

Bold page numbers indicate the presenter