Society of Electron Microscope Technology

Virtual Meeting

Wednesday 9th December 2020

14.00 - 17.00

SEMT Virtual Meeting 2020

Acknowledgements
SEMT would like to thank the Royal Microscopical Society for kindly hosting the SEMT virtual meeting 2020 via Zoom.
We hope that we will be able to return to our One Day Meeting in December 2021.
We look forward to seeing you again there.
Stay safe and well.

Meeting registration

http://www.semt.org.uk/future-meetings.html

Programme for SEMT Virtual Meeting 2020

14.00 **Welcome and introduction**: **Chris Jones - SEMT Chair**

14.05 **Electron Microscopy in Public Health England in 2020 - "May you live in interesting times"**
Matthew Hannah, Public Health England

14.30 **Chemical imaging of buried interfaces in organic-inorganic structures using FIB-ToF-SIMS, and applications for 2D materials**
Barry Brennan, National Physical Laboratory

14.55 **Optimised Sampling for (S) TEM Observations of Beam Sensitive Materials and Processes**
Nigel D. Browning, Department of Mechanical, Materials & Aerospace Engineering, Liverpool University

15.20 **SEMT AGM and comfort break**

15.40 **Probing chemical pathways in polyamide reverse osmosis membranes**
Alex Porter, Department of Materials, Imperial College London

16.05 **Investigating the effects of chemotherapy using aberration-corrected STEM**
Alex Sheader, Department of Materials, University of Oxford

16.30 **Nanoflights : A Synoptic View on Microstructures in the SEM**
Stefan Diller, Scientific Photography, 97072 Wuerzburg, Arndtstrasse 22, Germany

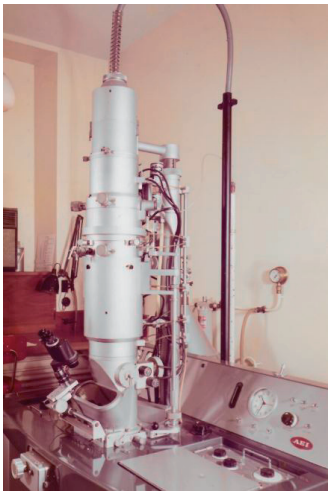
17.00 **Social time and discussion ' bring your own drink'**

18.00 **Meeting close**

Electron Microscopy in Public Health England in 2020 “May you live in interesting times”

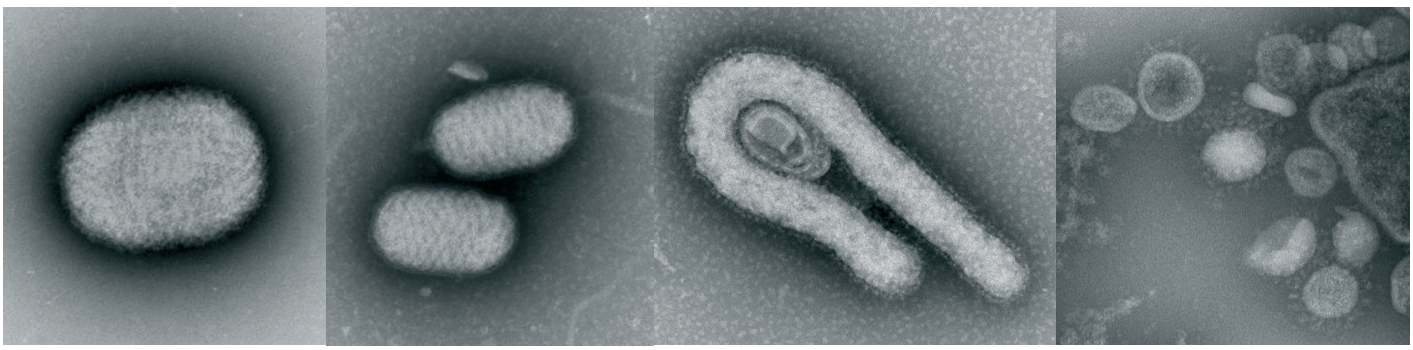
Matthew Hannah, High Containment Microbiology, National Infection Service, Public Health England

There have been electron microscopes within the UK’s public health laboratory system since the late 1960s with the Central Public Health Laboratory in Colindale buying its first electron microscope (an AEI 6B TEM) and appointing its first dedicated electron microscopist (Anne Field) in 1968. Historically, transmission electron microscopes were an important tool for clinical virologists as different families of viruses have distinct morphologies in an electron microscope after negative staining and therefore this quick, simple and relatively robust method was adopted for primary diagnosis from clinical samples. At “peak electron microscope” in the 1970s there were three TEMs in the Virus Reference Department of the Public Health Laboratory Service (PHLS) labs that were being used by teams of scientists doing primary diagnostic and outbreak work -mostly for enteric viruses- as well as research and reference work for other virus groups.



Since the 1970s the public health laboratories have been re-structured and re-branded a number of times (with another one on the way), but thankfully, during this time and in spite of the revolution in molecular testing, electron microscopy has been retained. Specialist virus diagnosis and testing in PHE is now almost entirely molecular, and although our latest TEM still provides a UKAS accredited diagnostic service to the NHS for two pox viruses (orf and molluscum contagiosum), the majority of instrument time is used characterising negatively stained virus particles or virus-like particles (VLPs) from reference material and research projects.

Crucially, TEM retains an important public health role in the realm of emerging and high-consequence viral pathogens and deliberate release of bioterrorist agents. This role exploits the fact that negative stain, diagnostic



EM is an unbiased catch-all method that requires no agent-specific reagents (antibodies or nucleotide primers) in order to identify an unknown suspected viral pathogen (at least down to family level), but it is also dependent on an effective and safe interface between high containment (CL3 and CL4) microbiology and electron microscopy. The recent COVID-19 pandemic has been a useful opportunity to test and strengthen this relationship.

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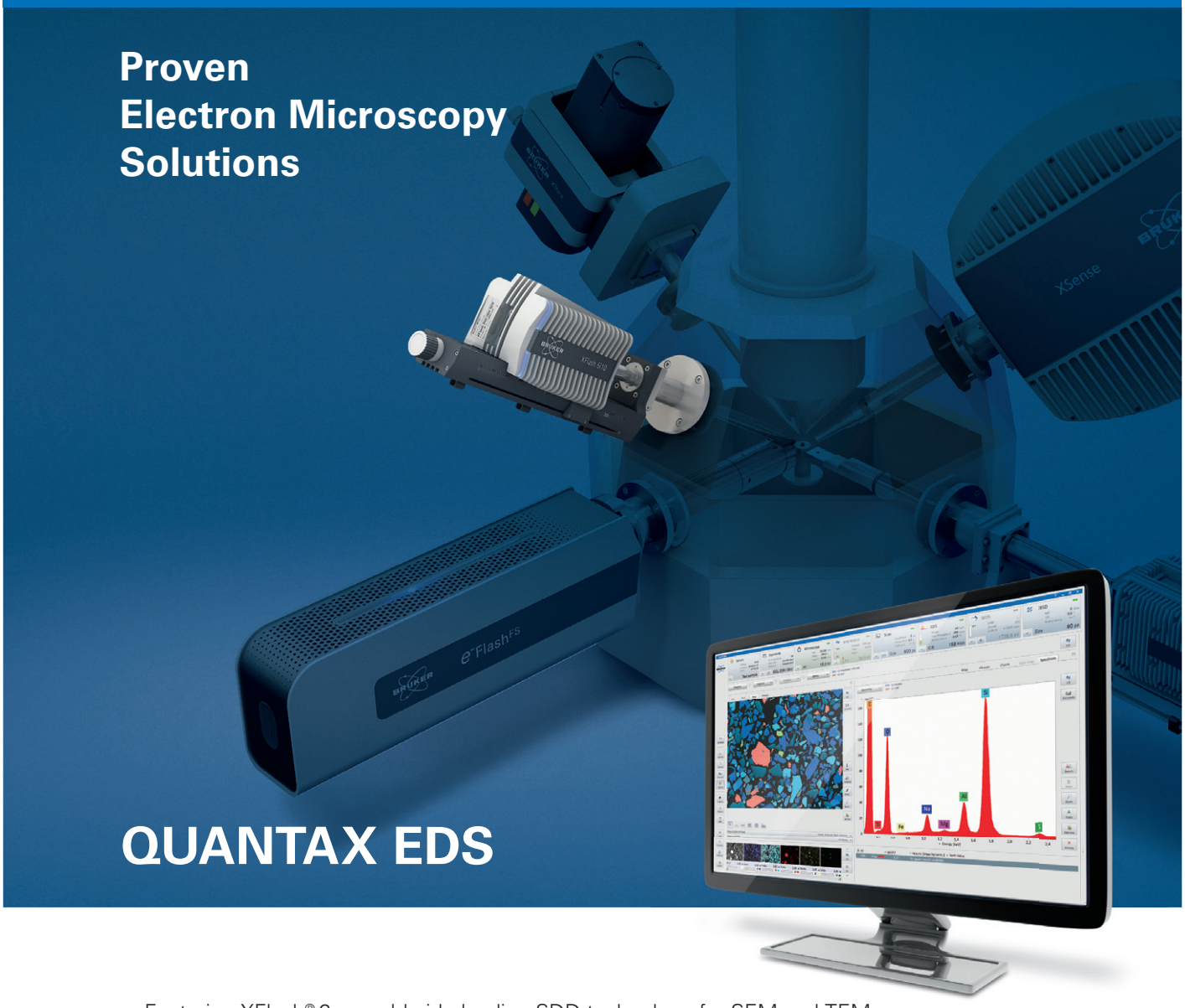

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Chemical imaging of buried interfaces in organic-inorganic structures using FIB-ToF-SIMS, and applications for 2D materials

Barry Brennan, Maria Vitalia Tiddia, Andrew Pollard and Ian S. Gilmore, National Physical Laboratory, Hampton Road, Teddington TW11 0LW

Organic-inorganic hybrid materials enable the design and fabrication of new materials with enhanced properties. The interface between the organic and inorganic materials is often critical to the device's performance and therefore chemical characterisation is of significant interest. Since the interfaces are often buried, milling by focused ion beams (FIB) to expose the interface is becoming an increasingly popular method to access these interfaces.

However, the FIB milling process rapidly damages the organic layers in these hybrid materials and as such an understanding of the processes involved in FIB milling and ways to recover signal from the damaged organic layer is needed and demonstrated. Chemical imaging of the milled sections can subsequently be obtained using time-of-flight secondary ion mass spectrometry (ToF-SIMS) which can provide high lateral resolution images, with detection levels of ppm and better, to allow visualization of the location of even trace levels of contamination or defects.

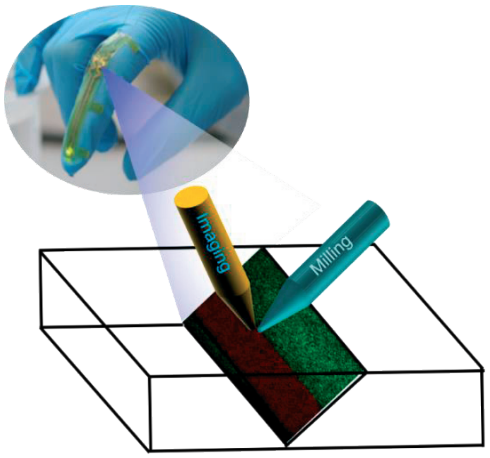
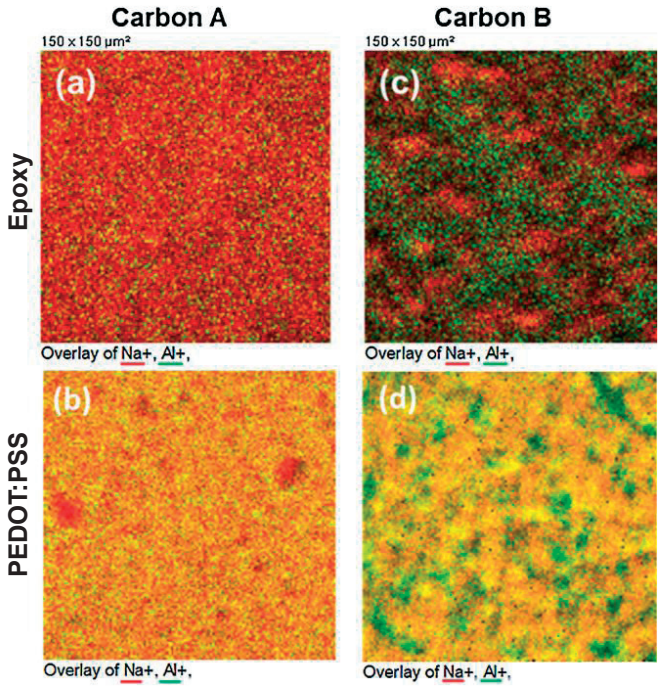


Figure 1. FIB-ToF SIMS of a strain sensor device showing imaging and milling beam orientation and revealing the organic(green)-inorganic(red) interface.



This talk will further discuss uses and applications of FIB milling, in conjunction with Argon Gas Cluster Ion Beam (GCIB) etching and ToF-SIMS imaging to provide an insight into the chemical distribution of 2D nanomaterials within layered devices and composite materials, in which the nanomaterials are dispersed to enhance the material properties.

Figure 2. Mapping of graphene dispersion within polymer composites: ToF-SIMS images of the graphene (red) and polymer (green) during 3D depth profiling with 2 different graphene materials in either Epoxy or PEDOT:PSS.

Optimised Sampling for (S) TEM Observations of Beam Sensitive Materials and Processes

Nigel D. Browning, [1,2*], B. Layla Mehdi[1,2], Daniel Nicholls[1], Mounib Bahri[1], Andrew Stevens[3]
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[2]Physical & Computational Science Directorate, PNNL, Richland, WA 99352, USA
[3]OptimalSensing LLC, Southlake, TX 76092. USA
e-mail: Nigel.Browning@liverpool.ac.uk

Images/spectra can now be routinely acquired from aberration corrected scanning transmission electron microscopes (STEM) to quantify the atomic scale structure, composition, chemistry, bonding, electron/phonon distribution and optical properties of nanostructures, interfaces and defects in many advanced materials. However, quantitative and reproducible atomic scale observations for some materials is actually harder with aberration corrected microscopes due to the increased beam current and the subsequent electron beam modification of the specimen during image/spectrum acquisition. This has led to a widely used definition of samples that "work" and samples where microscopy can't help – disenfranchising many important new materials from the benefits of high resolution electron microscopy. The challenge in developing and applying STEM methods is therefore now to focus on more efficient use of the dose that is supplied to the sample and to extract the most information from each image – reducing the beam effects (controlled by dose, dose rate and dose overlap) and broadening microscopy applications. Optimizing the dose/data content in non-traditional ways (i.e. not just simply lowering the beam current) achieves significant dose fractionation by reducing the number of pixels being sampled - allowing data to be automatically recorded in a faster, compressed form which is then de-compressed using inpainting methods. In this presentation, the basic approach to sub-sampling / inpainting / compressive sensing for dose control will be described. Results showing the use of in-situ liquid stages to study nanoscale dynamic processes will be presented and the potential insights that can be gained by increasing the image acquisition speed and/or controlling electron dose / dose rate / dose overlap for future research projects will also be discussed.

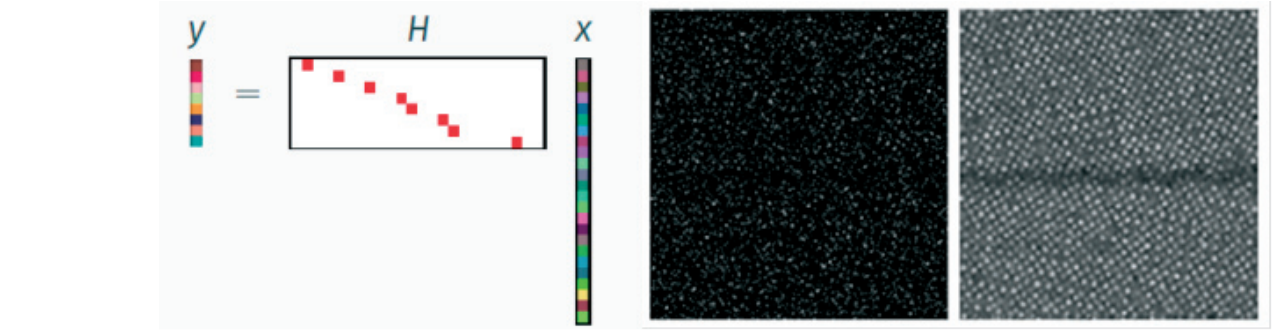

Figure 1: (a) the mathematical representation of sub-sampling is realized by (b) acquiring fewer pixels in STEM. The full image is reconstructed (c) using inpainting/compressed sensing methods.

Supported in part by the Chemical Imaging LDRD Initiative at PNNL for the U.S. DOE under Contract DE-AC05-76RL01830. This work was supported in part by the U.K. Faraday Institution Characterisation project FIRG013.

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Probing chemical pathways in polyamide reverse osmosis membranes

C.M. McGilvery[1], P. Abellan[2], M.M. Kłosowski[1], A.G. Livingston[3], J.T. Cabral[3], Q. M. Ramasse[2,4] and **A.E. Porter**[1]

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[2] SuperSTEM Laboratory, SciTech Daresbury Campus, Daresbury, WA4 4AD, UK
[3] Department of Chemical Engineering, Imperial College London, London, SW7 2AZ, UK
[4] School of Physics and School of Chemical and Process Engineering, University of Leeds, Leeds, LS2 9JT, UK

Reverse Osmosis (RO) membranes are widely used for sea water desalination applications. As the cost of installing desalination plants at sea is high it has become important to have a fundamental understanding of how the membranes work so that costs can be reduced. Much effort has gone into understanding the bulk properties of the membranes, but little effort on understanding then nanoscale interactions that control ion selectivity. The membranes are made from a polyester backing layer, a polysulfone (PSf) support and a polyamide (PA) membrane which is typically 100-500nm thick in a commercial membrane. Due to the membrane's complex hierarchical structure, the controllability of ion selectivity remains unclear. Recent work has suggested that although complex, the structure is actually made out of a single sheet of membrane about 10nm thick that has been 'crumpled'. There is also evidence that the top and bottom surfaces of the membrane are terminated with different functional groups suggesting that ion permeation pathways across the membrane may be due to chemical variations. Because of the amorphous nature of the polymer it is impossible to visualize any physical or chemical pathways using conventional transmission electron microscopy (TEM) or scanning TEM (STEM). The only method that can be used to investigate variations in chemistry on the sub nanometre scale is spatially resolved electron energy-loss spectroscopy (EELS). This, however, presents its own set of problems in the form of electron beam damage, which needs to be carefully controlled (if not mitigated) to yield meaningful conclusions.

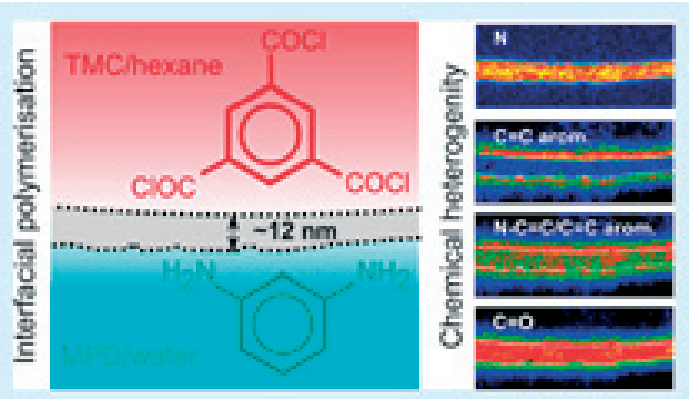

Figure 1: EELS spectrum images showing nanoscale chemical Heterogeneity in Aromatic Polyamide Membranes Applications prepared by an interfacial polymerization reaction[1]

Here we show that it is possible to use the N K-edge to map the active layer of a PA film using monochromated EELS spectrum imaging. The active PA layer is 12 nm thick, which supports previous neutron reflectivity data. Clear changes in the fine structure of the C K-edge across the PA films are measured and we use machine learning to assign fine structure at this edge. Using this method, we map highly heterogeneous intensity variations in functional chemistry attributed to N-C=C bonds within the PA. Similarities are found with previous molecular dynamics simulations of PA showing regions with a higher density of amide bonding as a result of the aggregation process at similar length scales. The chemical pathways that can be deduced may offer a clearer understanding of the transport mechanisms through the membrane.

Reference: McGilvery CM, Abellan P, Klosowski MM, Livingston AG, Cabral JT, Ramasse QM, Porter AEclose, 2020, Nanoscale chemical heterogeneity in aromatic polyamide membranes for reverse osmosis applications, ACS Applied Materials & Interfaces, 12: 19890-19902.

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Investigating the effects of chemotherapy using aberration-corrected STEM

Alexandra A Sheader[1,*], C Ferguson[2], S J L Flatters[2], R A Fleck[3], P D Nellist[1]

[1] Department of Materials, University of Oxford (UK) [2] Wolfson Centre for Age-Related Diseases, Institute of Psychiatry, Psychology & Neuroscience, King's College London (UK) [3] Centre for Ultrastructural Imaging, King's College London

An aberration-corrected scanning transmission electron microscope (STEM) is capable of resolving structures as small as a single atom [1]. Significant developments in instrumentation, data acquisition/processing, and in sample preparation techniques mean new information about many different materials is now within reach [2-4]. However, there are a number of practical concerns for acquiring high resolution STEM data on biological specimens - primarily limitations surrounding electron dose [5] and preservation of sample ultrastructure during the sample preparation process.

In this talk I will discuss how we have used high-resolution STEM to image single platinum atoms within a biological matrix. We have successfully demonstrated the use of high-angle annular dark field STEM to detect single platinum atoms from the chemotherapeutic oxaliplatin, within thin sections of rat dorsal root ganglia (see Figure 1). By applying quantitative HAADF methods we can perform zeptogram-scale weighing of oxaliplatin clusters in tissue, while preserving the sub cellular context of their location.

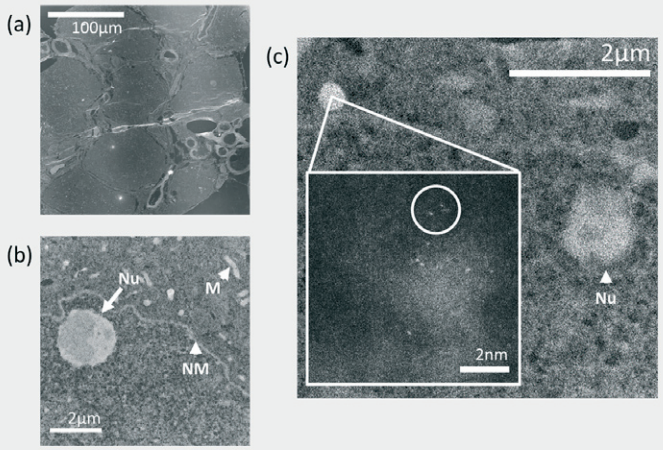

Figure 1: High-angle ADF images of DRG cells. (a) A low-magnification overview of DRG allows for identification of regions of interest. (b) Sections showed good ultrastructural preservation, with identifiable subcellular features such as mitochondria (M), the nucleolus (Nu) and nuclear membrane (NM) visible at higher magnifications. (c) Some regions in the DRG cytosol exhibit increased image contrast; further increasing the magnification shows single Pt atoms within these features.

In the second part of my talk I will discuss recent work we have performed towards quantifying elements of particular biological significance, such as Na, K and Ca. In particular, I will discuss challenges associated with acquiring electron energy loss spectroscopy (EELS) and energy dispersive X-ray spectroscopy (EDX) data of these elements by making measurements on a thin tissue sample to probe features within the cell. Using an approach based on experimentally measured partial scattering cross-sections, we

are able to acquire the mass sub-cellular features (such as mitochondrial CaPO storage granules, see Fig. 2) in units of atoms per nm2.

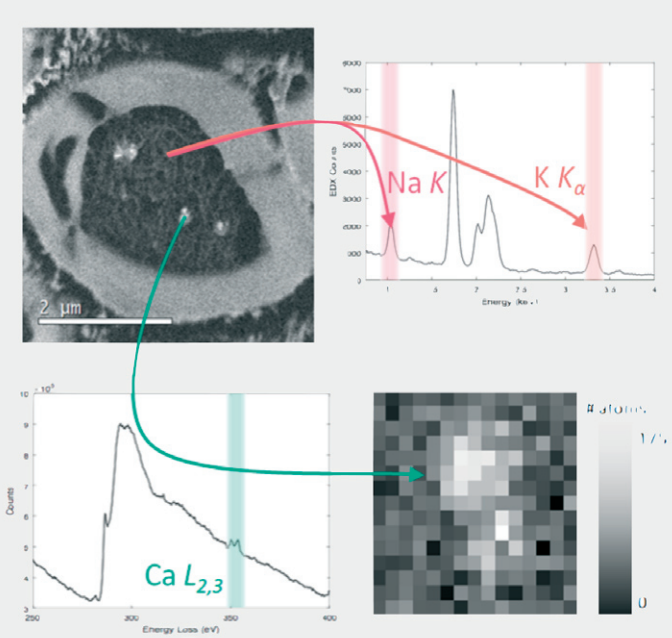

Figure 2: (top-right) Energy dispersive x-ray and (lower-left) electron energy loss spectra can be acquired from thin tissue sections. Both data can be converted into areal density (number of atoms per pixel) as shown here for the Ca $L_{2,3}$ EELS line.

[1] A V Crewe, J Wall and J Langmore. 'Visibility of Single Atoms', *Science* 168(3937) pp.1338-1340 (1970)

[2] G McMullan et al. 'Comparison of optimal performance at 300 keV of three direct electron detectors for use in low dose electron microscopy', *Ultramicroscopy* 147, pp. 156-163 (2014)

[3] L Kovarik et al. 'Implementing an accurate and rapid sparse sampling approach for low-dose atomic resolution STEM imaging', *Appl. Phys. Lett* 109, 164102 (2016)

[4] A Al-Moudi et al. 'Cryo-electron microscopy of vitreous sections', *EMBO J.* 23(18), pp. 3583-3588 (2014)

[5] R F Egerton. 'Mechanisms of radiation damage in beam-sensitive specimens, for TEM accelerating voltages between 10 and 300 kV', *Microscopy Research and Techniques* 75(11), pp. 1550-1556 (2012)

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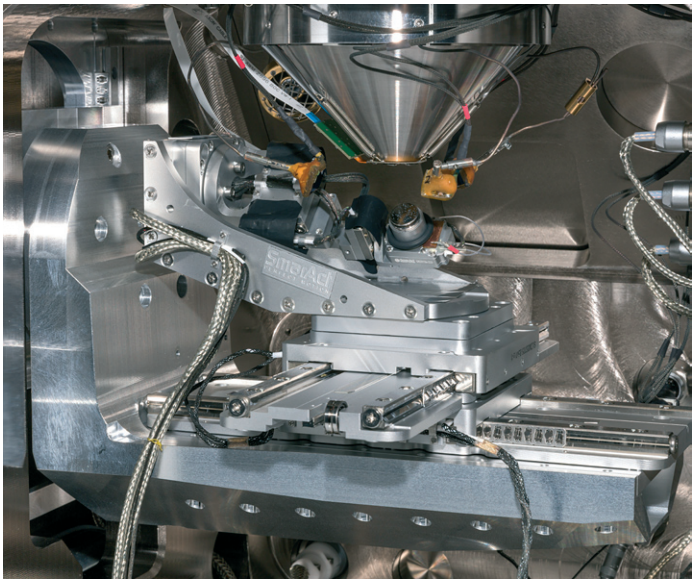
Nanoflights : A Synoptic View on Microstructures in the SEM

Technical and aesthetical aspects on multi-detector colour imaging, animated images in the Scanning Electron Microscope and development of the software nanoflight.creator

Stefan Diller - Scientific Photography, 97072 Wuerzburg, Arndtstrasse 22, Germany
Email: diller@stefan-diller.com; Websites: www.electronmicroscopy.info www.nanoflight.info

Every researcher should be committed to share the aesthetics of the hidden microworld with as many people as possible. This outreach to non-scientists and especially young people is essential in helping communicate basic and applied research to the general public. The ability of an individual image to inspire people inquiring about a hitherto unseen world is an important first step in establishing future generations of researchers and microscopists. Using colours to enhance the perception of and structures on the specimen is a powerful tool when employed to communicate imaging from a scientific source to people. A professional scientist or photographer must first search and acquire scientifically correct but also aesthetically appealing images. These rules aid in making an image accessible to a non-expert viewer, extending the impact of scientific imaging. An additional plus to create „eye-catchers“ is to add movement to microscopic view-fields, especially in scanning electron microscopy with its peculiar „light“ characteristics, its 3D image character due to signal generation and its extraordinary depth of focus. We developed the software nanoflight.creator which is used - together with available hardware - to set up „camera moves“ around microscopic specimen in a stable view-field from some millimeters down to ca 30 micrometers. The nanoflight sequence is programmed like a stop animation movie, defining waypoints, focus points, intensity levels and freely assigned colours to each available detector. To smooth out the intermediate frames a multi-dimensional Catmull-Rom spline routine is used for interpolation of the waypoint values.

My nanoflight work-horse: TESCAN MIRA3 FE-SEM with Spicer SC22 magnetic field cancellation system, XEI Evactron E50 and ThermoFisher NORAN SIX EDS



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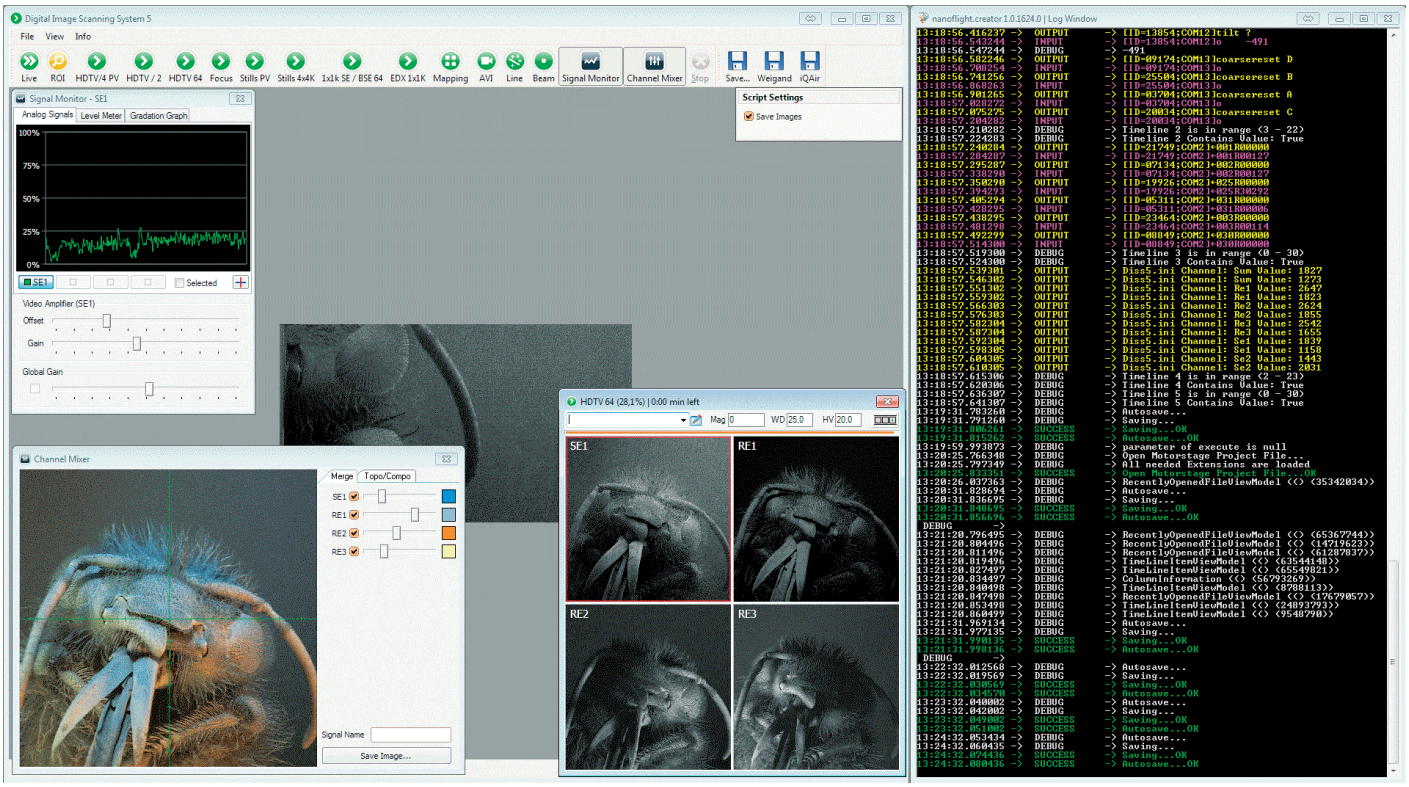

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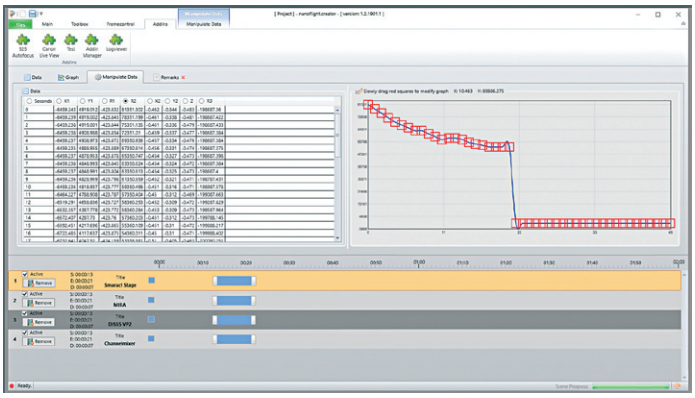
Finally the colour-mixed scans coming from the SEM are brought together in Adobe® AfterEffects, with additional colour and luminosity corrections, in some cases with aditional image stabilization and de-noising. Combined as a movie they yield the impression like flying around the microstructures with an eight degrees-of-freedom camera. Normally acquired in 2D, nanoflights are now possible in 3D using the 3D beam shift capabilities of the TESCAN MIRA3 FE-SEM to get pairs of stereo images with a deflection angle of some degrees in a view-field down to ca 50 µm and with good sharpness and astigmatism values to finally render a 3D SBS nanoflight without the need of tilting the stage, in that way facilitating the procedure and keeping the accuracy of the move. Hardware needed to set up „nanoflight“ sequences are: A large chamber scanning electron microscope; remote capability of most of the SEMs functions; an up-to-date multi channel image acquiring system specially modified to be accessed by script language; an eight axes piezo stage or any microscope stage with a very good accuracy and repeatability of coordinates; lot of detectors within the electron microscope to be able to choose the characteristic of „lighting“ and „coloring“ the specimen; the nanoflight.creator software package and for the hardware in use specially written „extensions“ to read / write values from / to all connected hardware.



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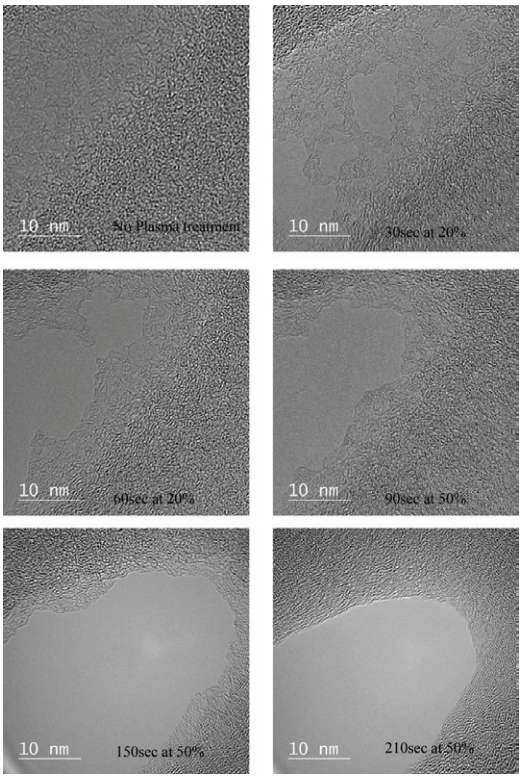

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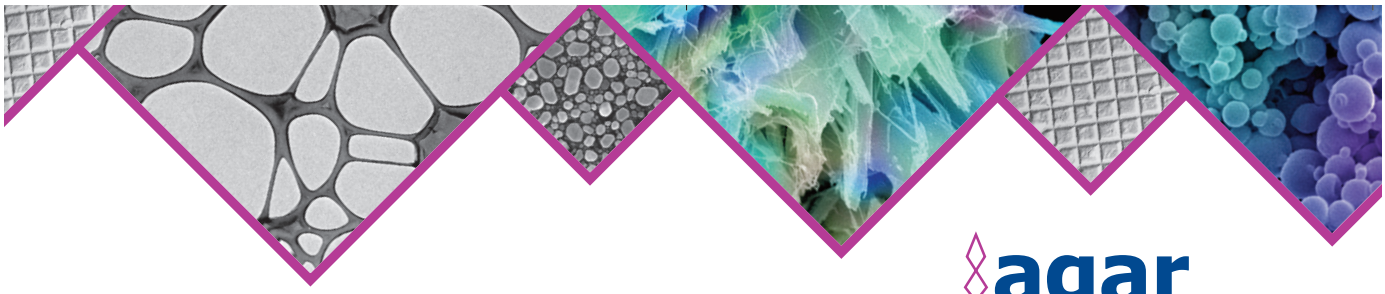

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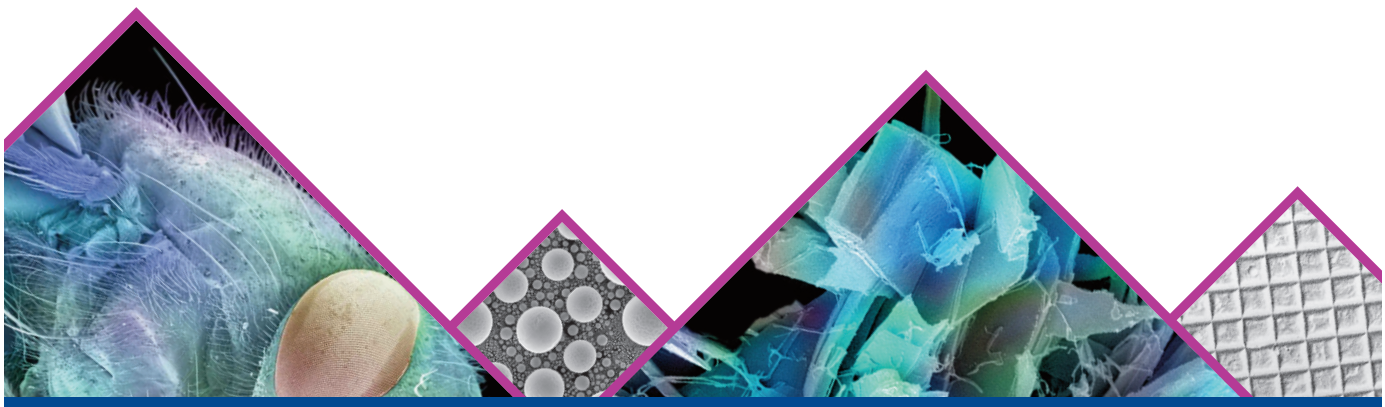

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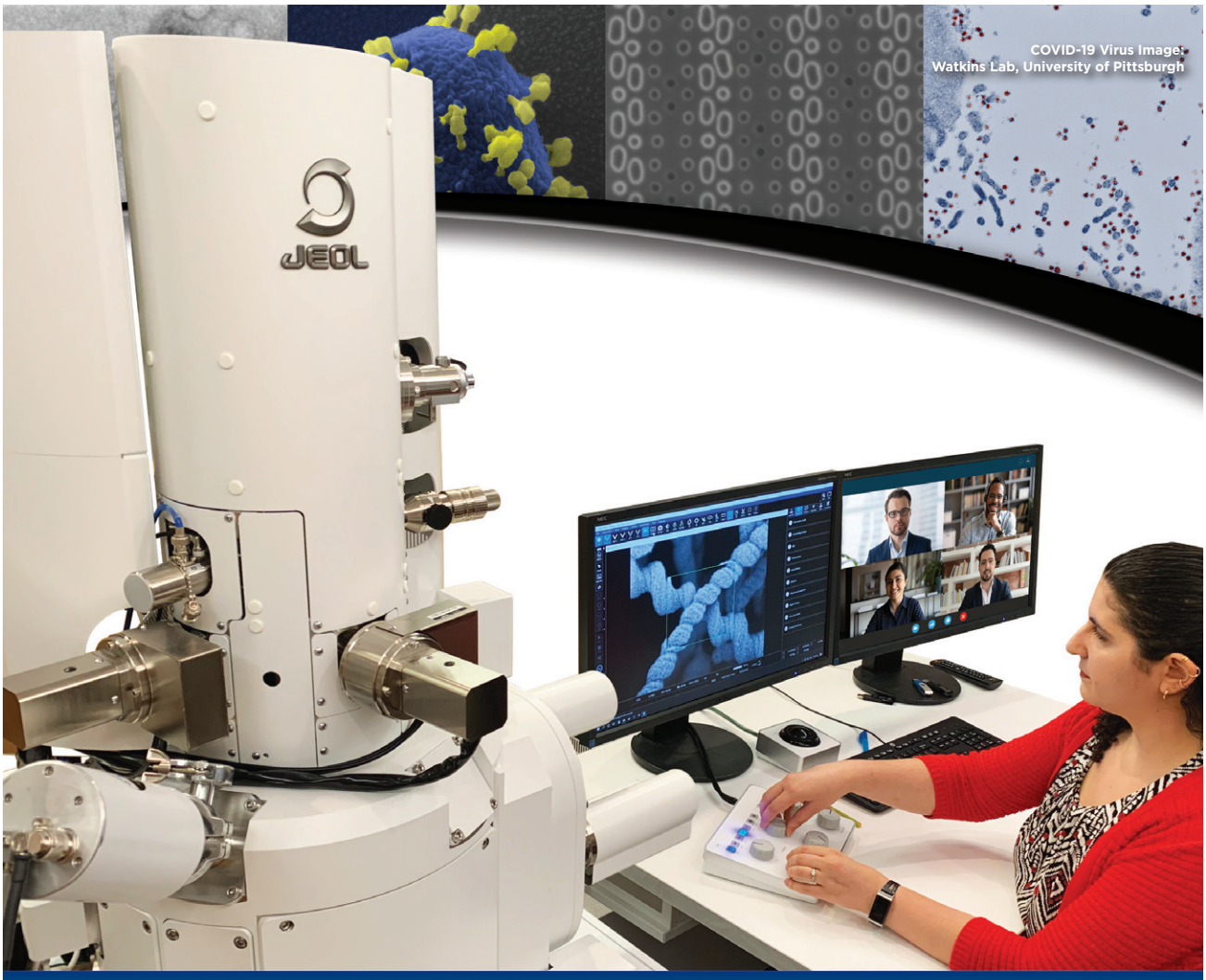

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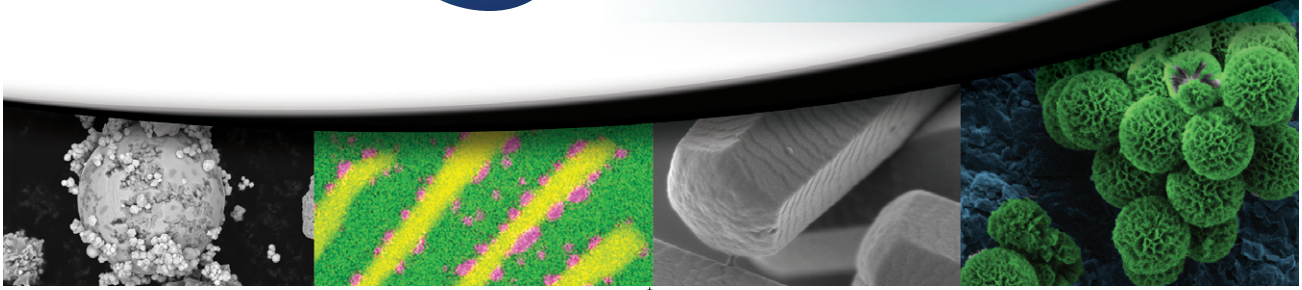

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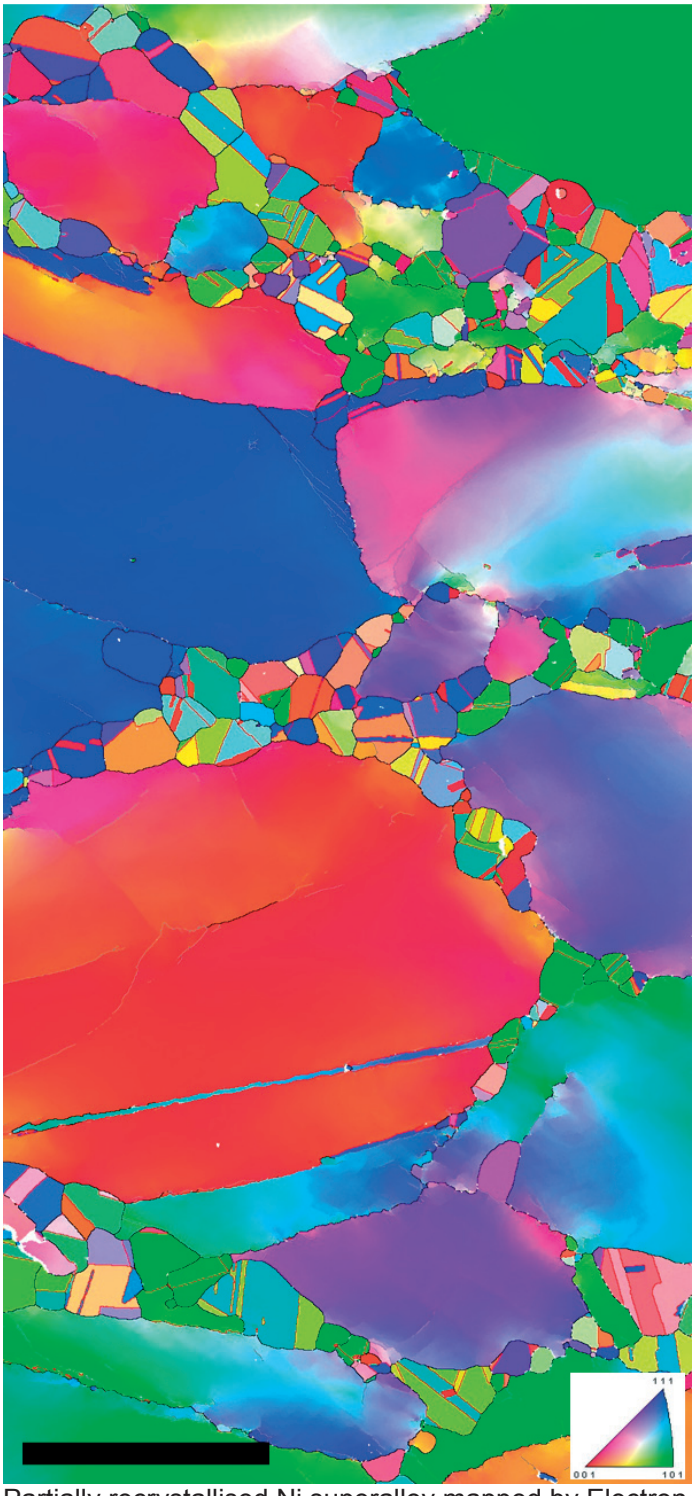
The SEMT

Affiliated to the Royal Microscopical Society, the Society of Electron Microscope Technology (SEMT) is a forum for ideas on techniques and applications in microscopy. It has become one of the foremost user groups in the country, addressing all aspects of microscopy from instrument design and specimen preparation to digital image acquisition.



Partially recrystallised Ni superalloy mapped by Electron Backscatter Diffraction to show grain orientation. Marker bar is 25µm. Courtesy Dr. Ken Mingard, NPL

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