Society of Electron Microscope Technology

One Day Meeting

Wednesday 14th December 2016

at

The Natural History Museum

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Acknowledgements

The SEMT wishes to express special thanks to The Natural History Museum as host of the One Day Meeting, and the following companies for supporting the trade exhibition:
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Programme for SEMT One Day Meeting

09.15 – **Registration** - Flett Theatre foyer

09.55 – **Welcome and Introduction**:
Chris Jones - SEMT Chair

10.00 **Mine site electron microscopy and the future of operational mineralogy**
James Strongman, Petrolab

10.30 **Electron beam techniques for the analysis of Fukushima-derived fallout**
Peter Martin, School of Physics, University of Bristol

11.00 **Coffee and Trade exhibition**

11.30 **RMS Beginners Competition** (10 minutes max each)

12.30 **SEMT AGM** – Flett Theatre

12.50 **Lunch and Trade exhibition** (+ judging for RMS competition and Conference delegates photograph – Flett Theatre

14.15 **The value of electron microscopy in diagnosing renal disease**
Robert Hangartner, Guy's and St Thomas' NHM Foundation Trust

14.45 **Electron tomography detects abnormalities in patients with Primary Ciliary Dyskinesia and 'normal ultrastructure'**
Amelia Shoemark, Royal Brompton Hospital (Imperial College)

15.15 **Tea and Trade exhibition**

15.45 **Making the practically impossible 'merely difficult' – cryogenic FIB lift- out for 'damage free' soft matter imaging**
Chris Parmenter, Nottingham Nanotechnology and Nanoscience Centre, University of Nottingham

16.15 **Metropilus: using electron microscopy to study type IV pili in Neisseria sp.**
Errin Johnson, Sir William Dunn School of Pathology, University of Oxford

16.45 **3D printing – making the impossible possible. An engineers perspective.**
Craig Cummings, Institute of Cancer Research

17.15 **Wine reception** - Flett Theatre foyer

19.00 – 22.00 **Dinner at NHM** + announcements and presentation to winners of the RMS Beginners competition

Mine-site Electron Microscopy and the future of Operational Mineralogy

James Strongman

Petrolab Limited, C Edwards Offices, Gweal Pawl,
Redruth, Cornwall, TR15 3AE

Automated mineralogy has been around for over 20 years driven by the development of QEMSCAN and MLA systems. These tools provide valuable mineralogical metrics including grainsize, liberation and association statistics. However, applying these in operational contexts has had to overcome three substantial hurdles. This has been (i) the presence of a sufficiently ruggedized system for mine-site deployment, (ii) slow turnaround times from sample preparation to reporting, and (iii) an over-reliance on off-site and often expensive specialists. This talk will cover a brief history of process mineralogy and look specifically at the ruggedized scanning electron microscope as a critical tool in the development of operational mineralogy.

Electron beam techniques for the analysis of Fukushima-derived fallout

Peter Martin

Interface Analysis Centre, HH Wills Physics Laboratory, University of Bristol, UK, BS8 1TL
Email: peter.martin@bristol.ac.uk

Peter Martin was awarded a BSc (2013) in Geology from the University of Bristol, UK and is currently undertaking his PhD within the Interface Analysis Centre in the School of Physics under the supervision of Prof. Tom Scott. Currently mid-way through his studies, Peter's work looks to undertake a highly detailed analysis on the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident and has such made several fieldwork expeditions to the radiation affected region. As well as the publication of work from within the theme of his PhD, Peter has also published more widely within the scientific literature on a range of materials problems through the application of electron microscopy and associated analytical techniques.

In March of this year, the incident at the Fukushima Daiichi Nuclear Power Plant in Japan reached its five-year anniversary. Tied with the 1986 events of Chernobyl as one of the worst nuclear disasters to have ever occurred, much is still to be known about the state and environmental behaviour of many of the contributing contaminants. These include the longer-lived, less radioactive, but more chemically toxic species, such as the actinides of uranium, neptunium and plutonium – through which mass-spectrometry methods have highlighted their presence in the vicinity of the plant (as well as much further afield).

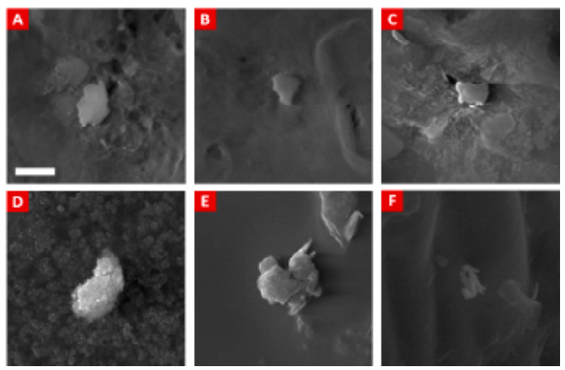

Figure 1 (A – F). SEM images showing uranium-containing particles under variable pressure conditions observed on the surfaces of moss and lichen material. Scale bars: 1 µm.

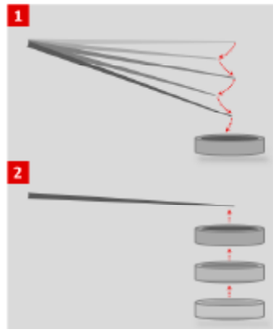

Figure 2. Sequence steps progressing the needle of the Kleindiek™ MM3-A Micromanipulator into contact with the sample via, (1) a saw-tooth motion lowering and subsequently extending the tip into eventual contact with the sample or (2) centring both the particle and needle under the electron-beam and raising the stage to the tip.

With nearly all of the work on the analysis of the radioactive material released from the multiple reactor-building event surrounding the short/medium-lived fission-product isotopes of cesium, this work centres on the analysis of sub-micron uranium particulate. Through the application of a low-vacuum SEM, micromanipulators and specialist electron-beam hardening adhesive, individual suspected fallout particles were identified and

subsequently isolated. Their removal facilitates a wide range of characterisation techniques to be performed, including TEM and synchrotron-radiation analysis.

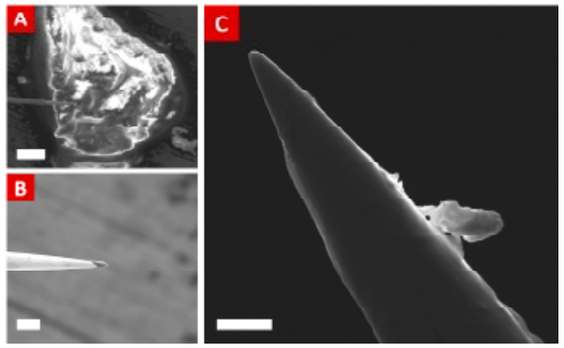

Figure 3. Steps detailing the removal of a particle from the containing bulk material, (A) application of SEMGlu™ to the top of a tungsten or glass needle, (B) progressive movement of needle into eventual contact with the particle, before increasing the beam-current to polymerise the adhesive to render the particle strongly attached to the end of the needle (C). Scale bars: 15 µm (A), 2 µm (B), 1µm (C).

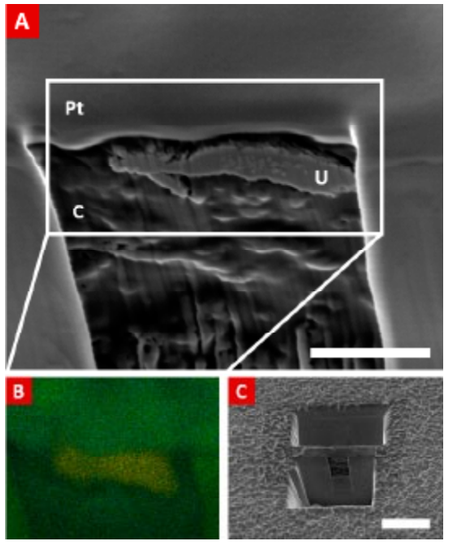

Figure 4. (A) Gallium ion-beam section through particle D to reveal its internal structure with platinum protective strip, uranium particle and underlying carbon mount identified, (B) EDS mapping of the particle to confirm distribution of uranium throughout sample and (C) continued ion-beam cutting to remove sample for TEM analysis. Scale bars: 1 µm (A and B), 5 µm (C).

RMS Beginners Competition

High contrast staining of plant endomembrane systems using ZIO and sample preparation for serial block face scanning electron microscopy

Jake Richardson[1],*, Louise Hughes[1], Chris Hawes[1]

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Imaging plant material using electron microscopy is improved by incorporating selective staining into sample preparation to provide high contrast to regions of interest. The endomembrane system of plants can be selectively stained using a zinc iodine/osmium tetroxide (ZIO) mix. The stain provides sufficient contrast and improves the conductivity of the sample resulting in a reduction of charging after prolonged periods under the electron beam. ZIO staining is incorporated when imaging using transmission electron microscopes (TEM) for ultrathin sections as well tomography, and more recently in the preparation of serial block face scanning electron microscopy (SBF-SEM) samples. Work has focussed on producing samples stained with ZIO which contain no precipitation artefacts allowing for accurate analysis of the images acquired. In tandem the development of staining protocols which shorten preparation time with the incorporation of microwaves has begun. Improvements in staining, preparation time and sample mounting protocols will increase productivity of the department by allocating more time for imaging using TEM and SBF-SEM.

Key words: endomembrane system, staining, mounting, ZIO, TEM, SBF-SEM

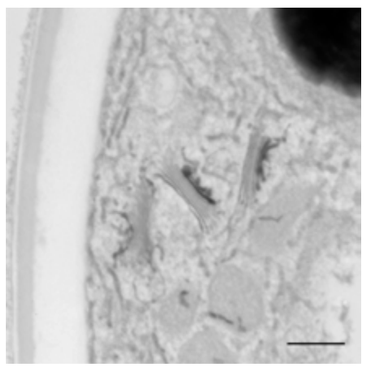


Figure 1 - 100nm thick section of venus fly trap (*Dionaea muscipula*) leaf stained with ZIO for six hours and embedded in Spurr resin with visible staining of Golgi bodies and the surrounding endoplasmic reticulum. Scale bar = 500nm.

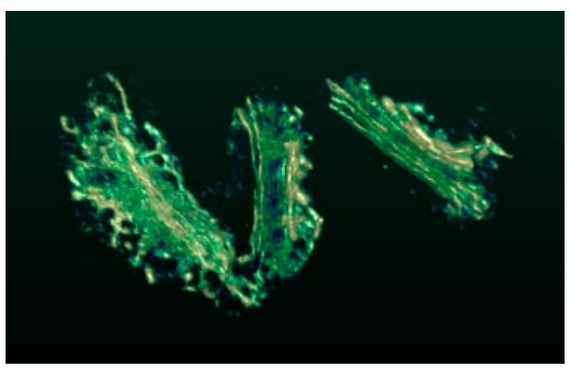


Figure 2 – Maximum intensity rendering of Golgi bodies (shown in Figure 1) using an aligned tomography stack taken from a 100nm section.

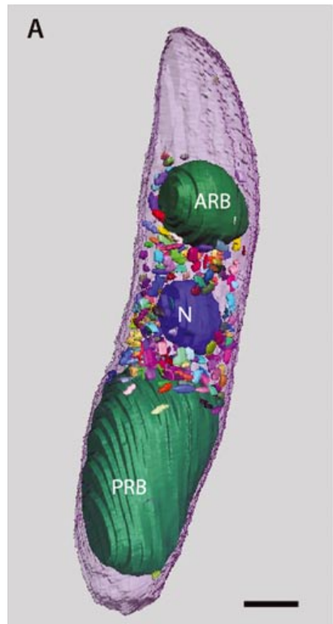
Quantitative analysis of the ultrastructure of *Eimeria tenella* and investigating organelle dynamics during development

Alana Burrell[1,2], Sue Vaughan[1], Virginia Marugan-Hernandez[2], Fiona Tomley[2]

[1] Dpt of Biological and Medical Sciences, Oxford Brookes University; [2] Dpt of Pathology and Pathogen Biology, The Royal Veterinary College, London

Since the 1960's, ultrastructural studies have played an important role in advancing our understanding of the complex biology and life-cycles of *Eimeria* parasites: protozoan organisms capable of causing enteric disease in a vast range of animals. With recent advances in high resolution instrumentation there is now the opportunity to expand on this body of existing knowledge. By using a novel three dimensional electron microscopy technique (serial block face – scanning electron microscopy) we were able to quantify organelle numbers and volumes for different developmental stages of *Eimeria tenella*. As well as producing a model of the freshly hatched parasite, these data revealed some unexpected dynamics of a poorly understood class of organelle, the refractile bodies. These organelles were seen to decrease in number soon following invasion whilst retaining their total volume; observations which have stimulated further hypothesis led investigations regarding their role in *Eimeria* development. In an era where biological research is dominated by molecular based techniques, studies such as this show the continued relevance of imaging modalities such as electron microscopy. Further investigation at an ultrastructural level may be vital in answering some of the many outstanding questions about the biology of *E. tenella*.

Keywords: *Eimeria*, serial block face – scanning electron microscopy



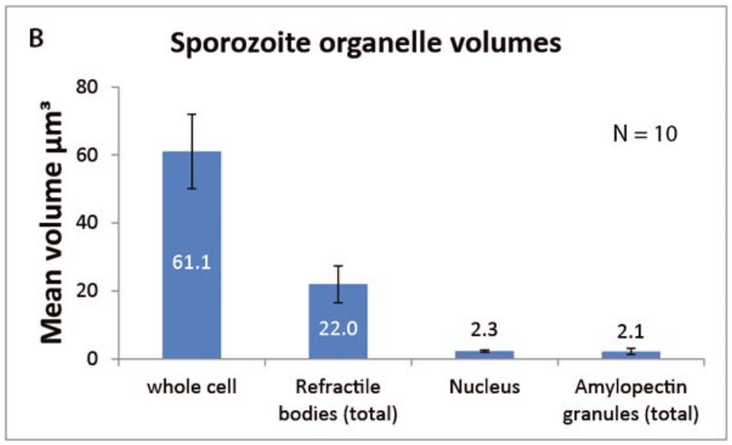


Figure 1: Freshly purified sporozoites were imaged using serial block face - scanning electron microscopy (SBF-SEM) and several organelles; nucleus (N), anterior refractile body (ARB), posterior refractile body (PRB) and amylopectin granules (multi-coloured) were modelled using AMIRA (FEI Visualization Sciences Group) (A). Scale bar ~ 2µm. Volumes were calculated from the modelled organelles (B): whole cell volume was 61.1µm³; nuclear volume was 2.3µm³; refractile body volume was 22µm³ and amylopectin granule volume was 2.1µm³.

Multiple Length-scale Imaging of Biomimetic Hierarchical Mineralized Materials

Sherif Elsharkawy[1,2,3*], Maisoon Al-Jawad[3,4], Nadezda V. Tarakina[2], Andy Bushby[2,4], Alvaro Mata[1,2]

[1]Institute of Bioengineering, Queen Mary University of London, United Kingdom. [2]School of Engineering and Materials Science, Queen Mary University of London, United Kingdom. [3]Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, United Kingdom. [4]Materials Research Institute, Queen Mary University of London, United Kingdom.
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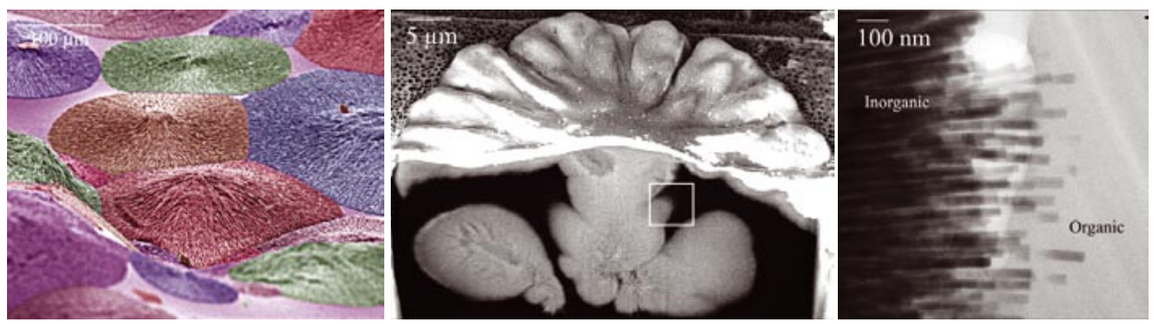
A major goal in materials science is to develop bioinspired functional materials that can offer precise control of building-blocks across multiple length-scales. Here we report a novel biomineralization system based on a tuneable organic-inorganic bulk environment that controllably nucleates and grows hierarchically-ordered apatite structures as coatings or membranes with remarkable multi-scale organization. The structures exhibit elongated apatite nanocrystals of about 85±22 nm in cross-section that are aligned and organized into approximately 3.8±0.9 μm thick prisms that resemble those found in dental enamel. These prisms assemble further into circular structures hundreds of microns in diameter that come together to fill macroscopic areas. The hierarchical structures can be grown in the presence of the organic matrix as thin mineralized membranes or coatings over irregular rough surfaces. We used a comprehensive suite of advanced multi-scale imaging techniques, including TEM, FIB-SEM, FEGSEM, to investigate the mechanism of formation and its relation to the distinctive structure at multiple length-scale ranging from crystallographic, to nano-, to micro, and up to the macro-scale.



Images showing the hierarchical organization of the biomimetic mineralized material. Using FIB-SEM, the structures tend to have a root-like structure at the centre, which exhibits an intimate organic-inorganic relationship.

Spatial and temporal progression of human incisal enamel biomineralisation

M. Al-Mosawi[1], G.R. Davis[1], A. Bushby[2], J. Montgomery[3], J. Beaumont[4] and M. Al-Jawad[1].

[1] Centre for Oral Growth and Development, Institute of Dentistry, Queen Mary University of London, London, E1 4NS, UK. [2] School of Engineering and Materials Science, Queen Mary, University of London, London, E1 4NS, UK. [3] Department of Archaeology, Durham University, Durham, DH1 3LE, UK. [4] Department of Archaeological Sciences, University of Bradford, Bradford, BD7 1AZ, UK.
Keywords: Enamel, Biomineralisation, X-Ray Microtomography, Quantitative Backscattered Electron Imaging, Synchrotron X-Ray Diffraction

Precise timing and spatial progression of human enamel biomineralisation are largely unknown due to scarcity of developing human enamel specimens for research. This information is crucial for optimising biomimetic regenerative/reparative dentistry routes. The aim was to spatially characterise the crystallography, mineral concentration and microstructure of incisal enamel at various developmental stages.

Four immature incisors were obtained from archaeological sources. A type-matched fully-developed tooth was used for comparison. X-ray microtomography (XMT) with 15μm3 resolution at 90kV, quantitative backscattered electron imaging (qBSE) operated at 5kV with 10mm working distance and synchrotron X-ray diffraction (S-XRD) were optimised to map mineral concentration (gcm-3), microstructure and crystallites organisation respectively.

XMT and qBSE revealed that mineral concentration increases with enamel maturation. They also confirmed that mineralisation progresses cervically and peripherally as enamel matures. qBSE and S-XRD showed that prisms (≈3-8μm) run approximately parallel to each other near the surface whereas towards inner enamel they divide into two distinct groups which deviate in direction, with one group exhibiting higher crystallite organisation. Furthermore, qBSE revealed that prismatic enamel mineralises before the interprismatic enamel.

These results provide new insights into the understanding of the natural growth of human enamel, and would facilitate the development of reparative/regenerative biomimetic dental strategies.

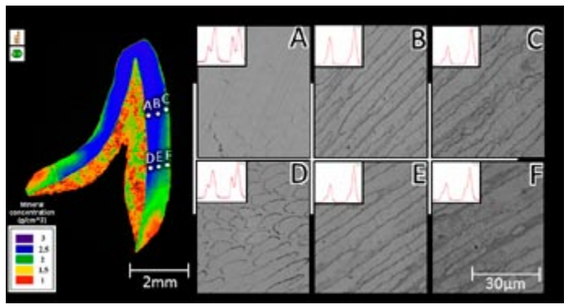


Fig: XMT mineral density map of a developing incisor with 6 selected regions. qBSE shows the prisms deviating in two direction in inner enamel (A & D) corresponding with 4 pronounced peaks in the azimuthally integrated (0 0 2) reflections from S-XRD.

Ultrastructural studies of the outer retina in murine models – exploiting novel 3D imaging technology

Eloise Keeling[1], Nicole Tan[1], David Chatelet[2], Patricia Goggin[2], Anton Page[2], Arjuna Ratnayaka[1]

[1] Clinical and Experimental Science, Faculty of Medicine, University Hospital Southampton, UK
[2] Biomedical Imaging Unit, University Hospital Southampton, UK

Background: Age-related Macular Degeneration (AMD) is a degenerative disease of the macula, the central part of the retina responsible for focused vision. AMD leads to irreversible sight loss and is caused by changes to tissues of the outer retina. Thus far, pathological changes in these tissues have only been observed and studied in 2D[1]. For the first time, we have utilised a novel imaging technology to reconstruct the mouse outer retina in 3D. Once this technique has been optimised, we will be able to study structural changes in donor human eyes across different stages of AMD.

Methods: The posterior pole of perfusion-fixed wildtype C57BL/6 mice eyes were embedded in Spurr resin for Serial Block-face Scanning Electron Microscopy (SBEM) following an adapted Ellisman protocol[2]. Ultra-thin sections were taken and viewed by conventional 2D transmission electron microscopy to identify the area of interest and ensure good tissue preservation. A 500µm^3 trapezium was cut out and mounted on an aluminium pin and loaded into a Gatan 3-View microscope. 50nm thick sections were taken for the entire depth of the sample generating a 3D stack of the outer retina. We focused on the RPE layer, the primary site of AMD pathology1. Single RPE cells were identified and segmented using the Fiji plugin 'TrakEM2' and reconstructed in 3D using Amira.

Results: Measurements of BrM and the RPE Microvilli were taken every 50 slices within the image stack. It was found that the average length of RPE microvilli remains at ~5µm irrespective of the position of the RPE cell in the retina (central retina: 5.52µm; peripheral retina: 5.15µm). Thickness of the supporting BrM was 408.96nm in the peripheral retina and slightly thinner in the central retina 365.82nm. These measurements correspond to BrM thickness changes between the central and peripheral retina previously reported[3]. Due to the high proportion of bi-nucleate cells in the mouse central retina3, both a bi-nucleate and a mono-nucleate cell were segmented. Interestingly, both nuclei in the bi-nucleate cell have similar dimensions to that in the single-nucleated RPE cell. 3D analysis demonstrated that the nucleus in the single-nucleated cell was 139.28µm^3 whilst the two nuclei in the bi-nucleate cell were 143.23µm^3 and 155.98µm^3 respectively. The volume of the single-nucleated cell (excluding nuclei, microvilli and basal infolds) was 1183.67µm^3 whilst the volume of the bi-nucleate was 2114.00µm^3.

Discussion: For the first time, we have been able to analyse structural elements of the RPE cells in 3D. This includes comparing and contrasting single and bi-nucleated cells which reveals that although bi-nucleate RPE cells are prevalent in the central retina, there appears to be no change in the size of the nuclei between the bi-nucleate and single nucleate cells. For the first time, we also have a realistic measurement of the RPE cell volume, with the bi-nucleate cell having a cytoplasmic volume almost double that of the single-nucleated cell. The novel data provides insights into how the RPE cells are organised in the outer retina. Current work on the microvilli and basal infolds are expected to provide a full picture of RPE cells in 3D. In the future, we also plan to segment the photoreceptors and choroid in order to understand its relationship with the RPE monolayer. We can then image donor AMD eyes and observe how structural arrangements change throughout different stages of AMD, thus providing insights into disease processes in the ageing human retina.

1. Bhutto I, Lutty G. Understanding age-related macular degeneration (AMD): Relationships between the photoreceptor/retinal pigment epithelium/Bruch's membrane/choriocapillaris complex. Molecular Aspects of Medicine 2012;33(4):295-317.
2. Deerinck TJ, Bushong EA, Thor A, et al. NCMIR methods for 3D EM: a new protocol for preparation of biological specimens for serial block face scanning electron microscopy. Microscopy 2010:6-8.
3. Volland S, Esteve-Rudd J, Hoo J, et al. A comparison of some organizational characteristics of the mouse central retina and the human macula. PLoS One 2015;10(4): e0125631.

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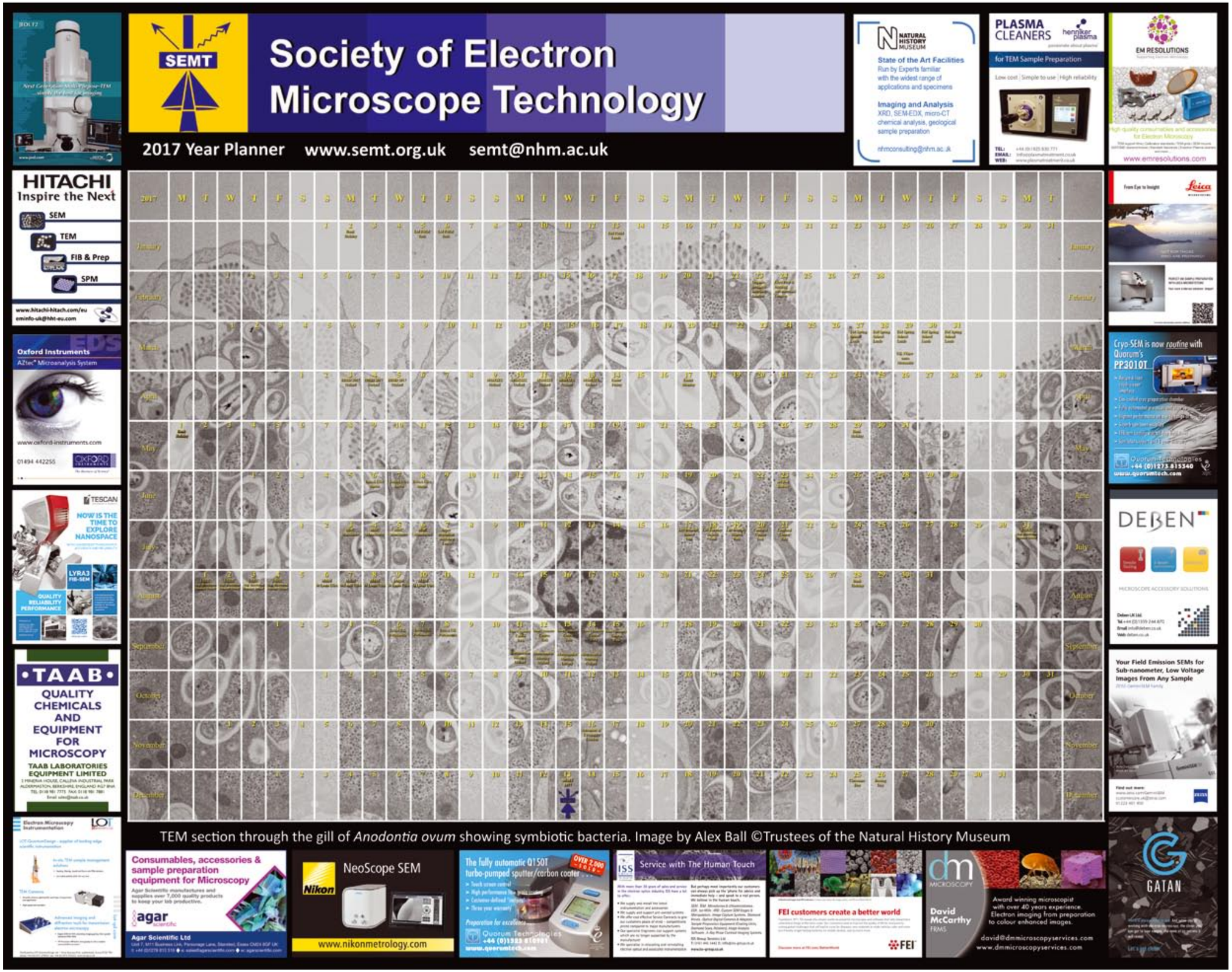

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The value of electron microscopy in diagnosing renal disease

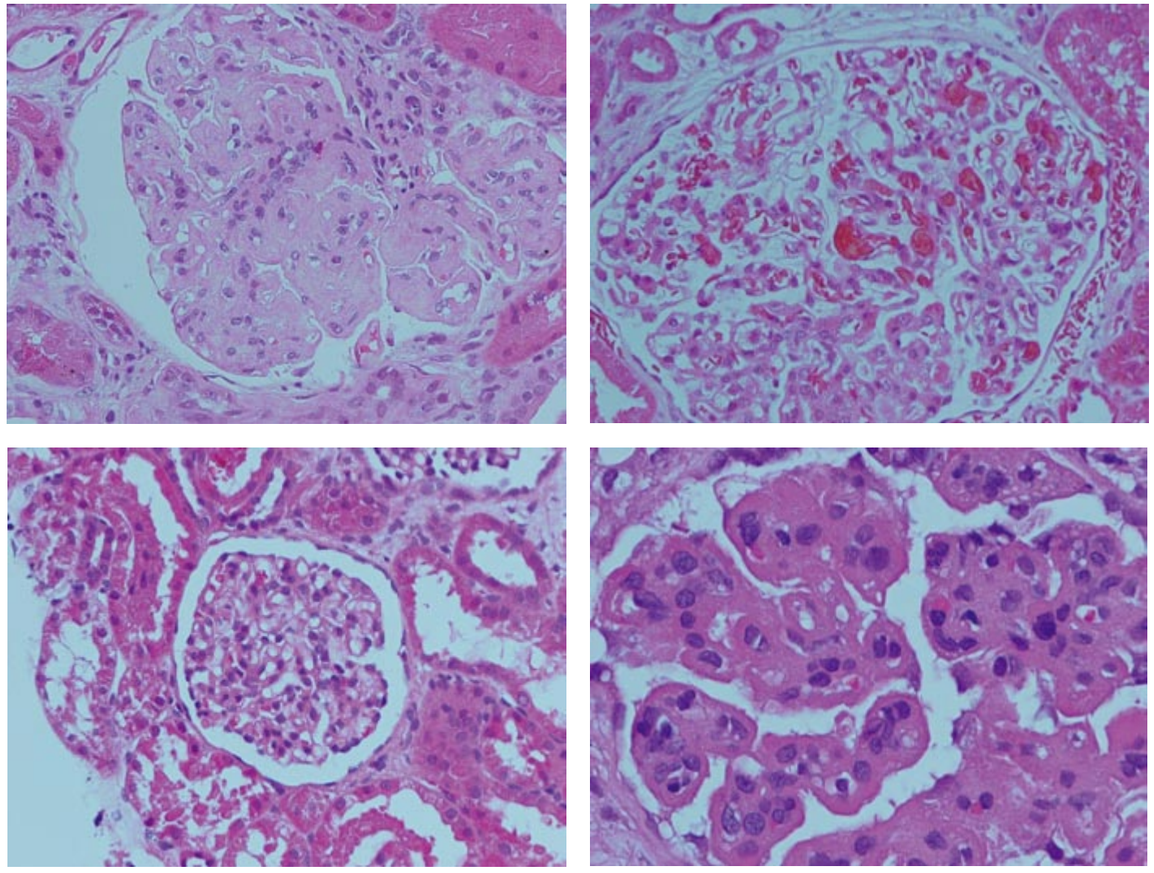
Robert Hangartner,

Guy's and St Thomas' NHM Foundation Trust:

In the era of molecular markers and genetics it is tempting to suggest that ultra-structural examination plays very little part in diagnostic pathology.

As I intend to illustrate and discuss, nothing could be further from the truth.

Electron Microscopy was essential to the diagnosis in these four cases.



Electron tomography detects abnormalities in patients with Primary Ciliary Dyskinesia and ‘normal ultrastructure’

Amelia Shoemark

EM unit, Department of Paediatrics, Royal Brompton Hospital, London, UK

Rationale: Primary Ciliary Dyskinesia (PCD) is a genetically heterogeneous condition where dysfunction of motile cilia results in chronic respiratory disease. Ciliary ultrastructure examined by Transmission Electron Microscopy (TEM) is usually used to confirm a diagnosis of PCD. However, 15-30% cases of PCD have apparently normal ciliary ultrastructure, making diagnosis difficult. The electron tomography technique, an extension of TEM, produces high resolution 3D ultrastructural models. The aim of this study was to determine if electron tomography can be performed on diagnostic material to detect ultrastructural abnormalities in patients with PCD and ‘normal ultrastructure’.

Methods: Longitudinal and transverse sections of proximal cilia were examined from araldite embedded nasal brush biopsies. 13 patients with PCD and 6 healthy controls were studied. Dual axis tomograms were collected on a Jeol 1400+. The data were analysed and averaged using IMOD and PEET software.

Results: Electron tomography indicated deficiency in the outer dynein arm volume (n=7), absence of central pair projections (n=3) and partial absence of nexin link structures (n=3) in patients with PCD and previously reported ‘normal ultrastructure’.

Conclusion: Electron tomography on diagnostic samples is effective in visualising defects which are difficult to see using standard TEM and could be used to confirm a diagnosis.

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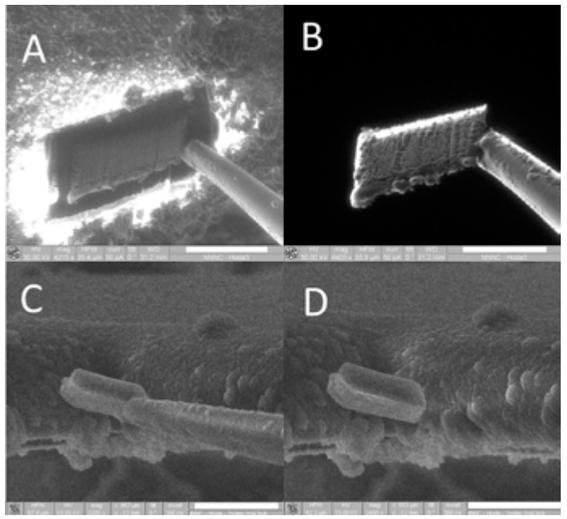
Making the practically impossible ‘merely difficult’ – cryogenic FIB lift- out for ‘damage free’ soft matter imaging

Chris Parmenter

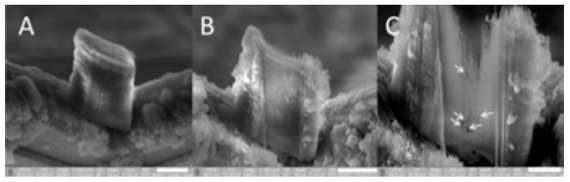
Nottingham Nanotechnology and Nanoscience Centre (NNNC), University of Nottingham
e-mail: Christopher.Parmenter@nottingham.ac.uk

One method to preserve high water content samples (cells, tissues, plant samples, suspensions, gels and food products) in their native state is cryogenic fixation, In the case of all but plunge freezing of small objects in thin vitreous layers, some sort of microsampling must be conducted to isolate the region of interest for TEM analysis. Cryo-ultramicrotomy, cryo-Focussed Ion Beam Scanning Electron Microscopy (FIBSEM) or cryo-SEM (via freeze fracture) are options to view internal structures. The preparation of thinned lamellae from bulk samples for Transmission Electron Microscopy (TEM) analysis has been possible in the Focused Ion Beam Scanning Electron Microscope (FIB-SEM) for over 20 years via the in situ lift-out method. Lift-out offers a fast and site specific preparation method for TEM analysis, typically in the field of materials science.

In order to enable cryo-lift-out, a number of technological and sample handling issues had to be overcome for routine application to cryo-preserved samples. This work presents the successful lift-out of high-water content lamellae, under cryogenic conditions (cryo-FIB lift-out) for those in pursuit of label- and damage-free information of soft and biological samples. Strategies are explored for maintaining cryogenic conditions, grid attachment using cryo-condensation of water and protection of the lamella when transferring to the TEM.



Lift-out using the cooled-probe (A) and the lamella secured with cryo-condensed ice (B). SEM images following the deposition of a lamella (A) the lamella secured to the grid using cryo-condensation of water (B). Scale bars are 20 µm



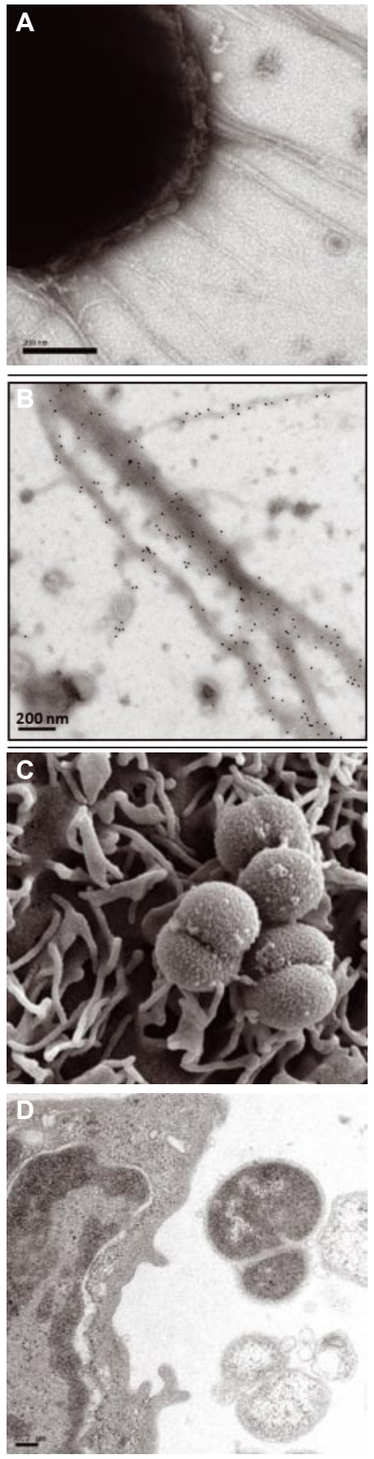
Cryo-FIB milling of a hydrogel samples to prepare electron transparent lamellae. Arrows indicate holes, pores or ultra-thin areas. (A, B) scale bar 10 µm (C) scale bar 5 µm.

Metropilus: using electron microscopy to study type IV pili in ***Neisseria sp***

Errin Johnson

Sir William Dunn School of Pathology,
University of Oxford

Neisseria meningitidis is a leading cause of bacterial meningitis, a devastating and potentially lethal disease that affects both children and adults. The bacteria use highly dynamic filamentous organelles called Type IV pili (Tfp) which mediate cell motility, the formation of micro-colonies and adhesion to the surface of epithelial cells in the human nasal tract. As part of a collaboration with the group of Christoph Tang at the Dunn School, we are using electron microscopy to characterise Tfp in several *Neisseria* species to better understand their role in the pathogenesis of bacterial meningitis and target new pathways for vaccines and treatment. I will discuss how we are applying EM to address specific questions, as well as some of the problems we have faced and how we overcame them. For example, in order to identify which pilin subunits are required for Tfp formation in *N. meningitidis*, *N. gonorrhoeae* and *N. cinerea* we used negative stain TEM, although, despite being a relatively straightforward technique, this first required extensive optimisation to preserve the Tfp in sufficient numbers for analysis.



Electron microscopy of *Neisseria sp.*: Tfp of *N. meningitidis* visualised using whole mount negative stain TEM (A), isolated Tfp immunolabelled with anti-pilin (B), SEM (C) and thin section TEM (D) of N. cinerea on mouse epithelial cells.

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3D printing. Making the Impossible Possible. An Engineers perspective

Craig Cummings

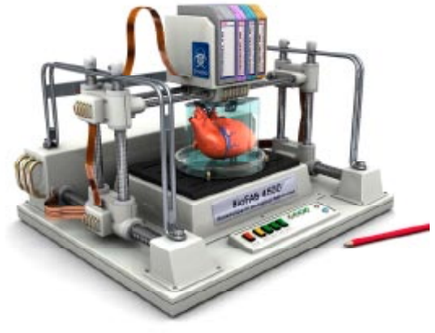
Institute of Cancer Research

A brief history of 3D printing

Types of 3D printer.

What can be printed?

The "AbdoMan" technology could transform radiotherapy



Who can print?

Projects I have been involved in.

3D printing in the medical environment.

The future ?????

The Society of Electron Microscope Technology

Notes

www.semt.org.uk

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The SEMT

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