Keeping you informed of the latest technology & techniques

Society of Electron Microscope Technology

SEMT
One Day Meeting

Wednesday 16th December 2009

at
The School of Pharmacy
University of London

Prospective members can be added to our Members List by contacting the Hon. Secretary Mr. David McCarthy, School of Pharmacy, Brunswick Sq. London WC1N 1AX

44 (0) 20 7753 5806;
Email: david.mccarthy@pharmacy.ac.uk.

The annual subscription is currently FREE.
Our web site : www.semt.org.uk

Officers

Chair
Mrs. Heather Davies

Secretary
Mr. David McCarthy

Treasurer
Dr. Nicola Mordan

Committee
Ms. Ann Dewar
Mr. David Robertson
Mr. Terry Cooper
Mr. Chris Jones
Dr. Alex Ball
Miss. Anne Drewe
Mrs. Trish Lovell
Mr. Derrick Lovell
Mrs. Hannah Armer
Dr. Tony Brain

Honorary Advisor
Mr. Chris Walker

Honorary Archivist
Dr. Jill Lewis

Acknowledgments
The SEMT wishes to express special thanks to The School of Pharmacy as host, and to the following companies for supporting the Trade Exhibition :

Agar Scientific Ltd
Deben UK Ltd.
Edax UK Ltd.
FEI UK Ltd.
Hitachi Scientific Instruments Ltd.
I.S.S. Group Services.
Leica Microsystems UK Ltd.
Jeol Uk Ltd.
Obducat Camscan Ltd.
Quorum Technologies Ltd.
Royal Microscopical Society
Taab Laboratories Ltd
Tron-Tech Ltd.
Zeiss SMT

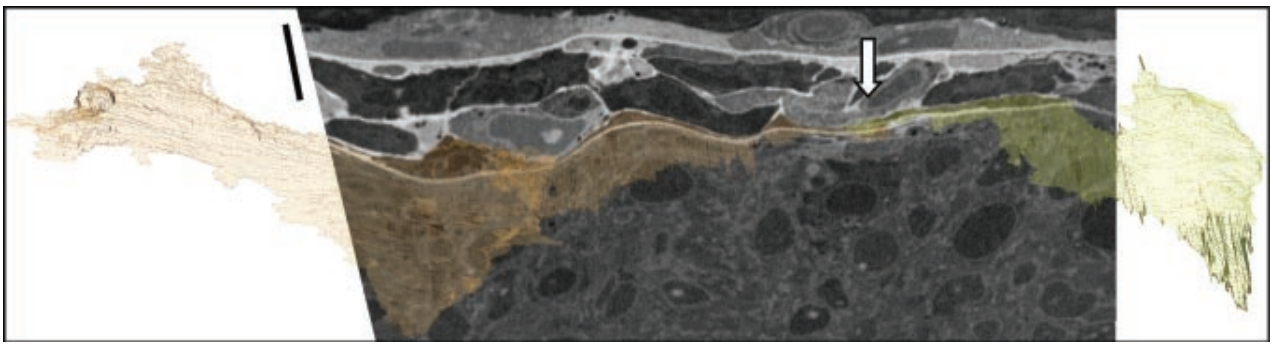
Future Programme : One day Meeting, 15th December 2009.

09.15	Registration, Tea/Coffee, Trade Exhibition
09.55	Introduction: Chair, Heather Davies.
10.00	**Correlative Volume EM – Finding the Needle in the Haystack.** **Dr. Lucy Collinson,** CRUK
10.35	**Characterisation of human embryonic stem cell lines and their neurodifferentiation potential.** **Dr. Roland Fleck**, NIBSC
11.10	Tea, Coffee, Trade Exhibition.
11.30	**RMS Beginners Competition**
	High durable anti-microbial surfaces and antibacterial properties evaluation in simulated hospital environment. **Yangchun Dong,** Birmingham University.
	Biosynthesis of Hydroxyapatite on Titanium Substrates. **Angi Wang**, Birmingham University.
	Correlative Light Electron Microscopy for the study of intracellular transport processes. **Edward Brown**, Bristol University.
	3-Dimensional Visualisation of the Intercalated Disc **Amanda Wilson,** KCL (Winner of the Don Claugher Bursay 2008)
12.30	Buffet Lunch, Trade Exhibition. Announcement of RMS Competition
14.30	**"What's in your barbecue charcoal?! A FE-SEM case-study from Pernambuco State, northeast Brazil"** **Dr. Caroline Cartwright,** British Museum.
15.05	**"The application of electron microscopy to the investigation of male infertility"** **Dr. Tim Ryder,** Charing Cross Hospital.
15.40	Tea, Coffee.
16.00	**"3D imaging in optically opaque materials: the structure of mudrocks"** **Stephen Wilkinson,** Imperial College.
16.35	**The use of TEM analysis to identify rock-dwelling cyanobacterium resistant to low Earth orbit** **Dr Karen Olsson-Francis,** Open University
17.10	AGM - Wine Reception
16.30	Conference Dinner

Correlative Volume EM – finding the needle in the haystack

Lucy Collinson
Cancer Research UK
London Research Institute

Progress has been made in the field of high resolution electron microscopy to the point where structural determination of single molecules is becoming routine. However, the challenge of obtaining high resolution structural data from bulk samples of cells, tissues and whole organisms remains largely unexplored. Recent innovations in Volume Electron Microscopy have led to a paradigm shift in high resolution imaging of large volumes of biological samples. These techniques exploit different sectioning methods to cut and image slices of material automatically, thus minimising artifacts and speeding up the process whilst freeing the operator to perform other work.
In general, samples are prepared as for traditional transmission EM. Much of the work in this area has been driven by neurobiology, where traditional EM cannot cope with the conflict between volume imaging of complex neural networks and high resolution imaging of neuronal connections.
We set out to develop and apply volume EM techniques to study another highly complex three-dimensional network, that of the developing circulatory system. The formation of new blood vessels, angiogenesis, is crucial in the patterning of the vascular system during vertebrate embryonic development in normal physiology and in pathological settings such as chronic inflammation, tumour progression and metastasis. Here we show that the point of fusion between growing blood vessels of transgenic zebrafish, identified in live confocal microscopy, can subsequently be traced through the structure of the organism using Focused Ion Beam/ Scanning Electron Microscopy (FIB/SEM) and Serial Block Face/ Scanning Electron Microscopy (SBF/ SEM). The resulting data give unprecedented microanatomical detail of the zebrafish, and for the first time allow visualization of the ultrastructure of a time-limited biological event within the context of a whole organism.



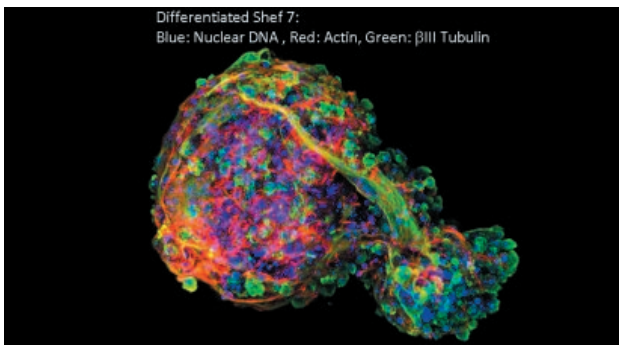
Zebrafish blood vessel endothelial cells undergoing fusion. 3D reconstruction of two adjacent endothelial cells (yellow, orange) are shown overlaying a CurvedSlice (Amira) through the original volume EM dataset. The white arrow indicates the contact point between the two cells, representing a volume of 3µm3 in a total volume of ~ 12,600,000 µm3 (the 'needle in the haystack').
Bar 5 µm (unidirectional).

Characterisation of human embryonic stem cell lines and their neurodifferentiation potential

Roland Fleck
Cell Biology and Imaging Division, National Institute for Biological Standards and Control
Blanche Lane, Potters Bar, EN3 6QG, UK

Keywords: human stem cells, embryonic, neurons, differentiation, epifluorescence.

Pluripotent human embryonic stem (hES) cells lines have considerable potential in regenerative medicine and for the production of functional cell substrates for bioassays. Their differentiation to functional endpoints, e.g., neurons, allows basic studies of cell commitment and development and provide an opportunities to evaluate cell function. The search for new therapies for neurodegenerative diseases and basic research into hES lines has resulted in a proliferation of differentiation strategies. This plethora of strategies and limited assessment of their performance limits the adoption of these techniques, thereby limiting their potential in establishing new bioassays. The principal challenges in developing a successful differentiation protocol are: a robust reproducible protocol, which produces high yield of a defined cell population; a minimum period to complete the differentiation process; and a protocol which provides cells with an "expected physiological function", e.g., release and uptake of neurotransmitters or an electrophysiological response. These challenges are compounded by the effect that inherent differences between human embryonic stem cell lines and their response to culture during the maintenance or differentiation conditions have on the differentiation end point.



In this study, human embryonic stem cells, Shef7, Shef3, HUES7 and WT4 were subjected to neurodifferentiation protocols. Maintenance of human embryonic stem (hES) cells in culture was performed with mitotically inactivated mouse embryonic fibroblast feeder layers (iMEF). The cells were split by manual and enzymatic passaging using trypsin (trypLE express). The differentiation protocols are based on treatment with retinoic acid (RA) or noggin. Retinoic acid protocol is employed for the differentiation of embryonal carcinoma cell line NTERA-2. Since then, many other studies with different cell types such as mesenchymal stem cells and neuroblastoma cell lines, adopted retinoic acid in their neural differentiation protocols. Noggin is bone morphogenic proteins inhibitor that promotes the conversion of ES cells to phenotypically stable neurons. Noggin has been mainly employed in stem cells differentiation protocols. hES cells were subjected to a five stage differentiation protocol. Briefly, cells were grown on iMEF prior to the formation of embryonic bodies (EBs). At day 3, EBs were plated under adherent conditions and then stimulated to differentiate with retinoic acid or noggin and fibronectin in serum-free media at day 4. At day 7, cells were incubated under serum-free conditions with bFGF from day 8 until day 14 and with cAMP until day 21. Immunocytochemistry (IC) and confocal microscopy were carried out to assess and characterise the neural differentiation at days 0, 3, 7, 14 and 21. Further characterisation was performed by quantitative RT-PCR. Confocal images were processed with Imaris software to reveal the 3D structure of hES colonies, EBs and neuronal networks. The complexity of the hES cells was revealed by cryo-EM.

Complementary studies are now in progress to evaluate what specific neuronal lineages are produced and the electrophysiological potential of differentiated cells.

RMS Beginers Competition

High durable anti-microbial surfaces and antibacterial properties evaluation in simulated hospital environment

Yangchun Dong
School of Metallurgy and Materials, University of Birmingham,

Outbreak of Hospital-acquired infections (HAIs), a large proportion of which are derived from contact transmission, stimulates development of inherently antiseptic materials. Nano-silver doping is one promising technique for relatively short term used dressings or polymers, but it is still a major challenge to ensure life-time antisepsis on surface of near permanently used metals. In this study an approach producing nano-bactericides located on the wear-resistant S-phase layer using novel active screen plasma (ASP) alloying technology may, apply for any stainless steel made instruments. Nano silver crystalline sized average 20μm was induced by plasma spattering. The compacting of S-phase as matrix increased hardness of substrate and prevents the wear of silver away from surface, thus offering sufficient amount of silver even in extreme friction conditions as determined from sliding wear assay. In vitro antibacterial test showed this nano silver integrated S-phase can achieve up to 93% reduction of Escherichia coli in short time (6 hours). The simulated long-term evaluation showed that nano-bactericides protected by the hardened S-phase layer can give rise to stable bactericidal behaviour over 120 times repeated devices cleaning cycles. We also found nanocopper embedded surface is more efficient than nanosilver contained surfaces to inhibit growth of Escherichia coli.

Figures and Captions (selected related to Microscopy technique)

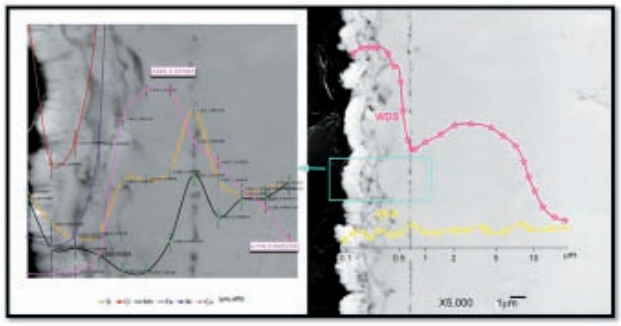


Fig.1 WDS results showed the element-depth profile in cross-section view including both trace element-Cu (A) and light element N (B). All lines are fitted from WDS point analysed data.

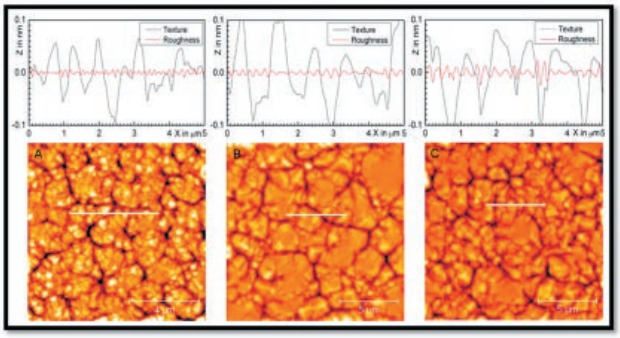


Fig. 3 AFM roughness profiles of stainless steel were obtained before anti-microbial test. The deflection roughness is mean roughness obtained from image area.

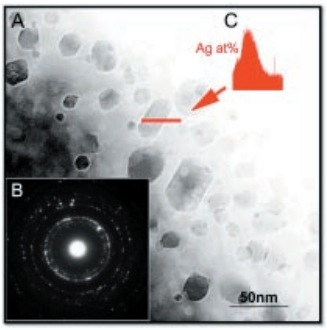


Fig.2 Nano-silver are encapsulated inside super-saturated S-phase con-firmed by TEM (A). Inset (B) shows the SAD pattern collected from this local area. Elemental mapping by scanning TEM (STEM) indicated the Ag varied from particles to S-phase matrix (C).

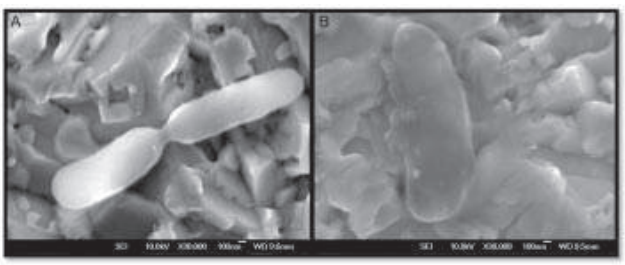


Fig. 4 Morphology of bacterial E. coli ON non-bactericidal samples (A) and silver embedded surface (B).

Biosynthesis of Hydroxyapatite on Titanium Substrates

Anqi Wang
University of Birmingham,

A novel method to coat titanium substrates, solid or porous, with hydroxyapatite (HA) for potential orthopeadic and dental applications via bacteria Serratia N 14 was utilised in this study. Biofilm was grown on the titanium supporting material and nano to micro scale calcium phosphate crystals formed on the substrate surface in an environment containing calcium chloride and an organic phosphate source. Heat treatment was applied to either calcine or sinter the crystals. SEM analysis showed that the crystal morphology changed as the crystal layer thickened, with EDX confirming that Ca/P ratio differed in parallel. XRD analysis confirmed that the crystals were hydroxyapatite. FTIR and TEM analysis is in progress to further characterise the crystals.

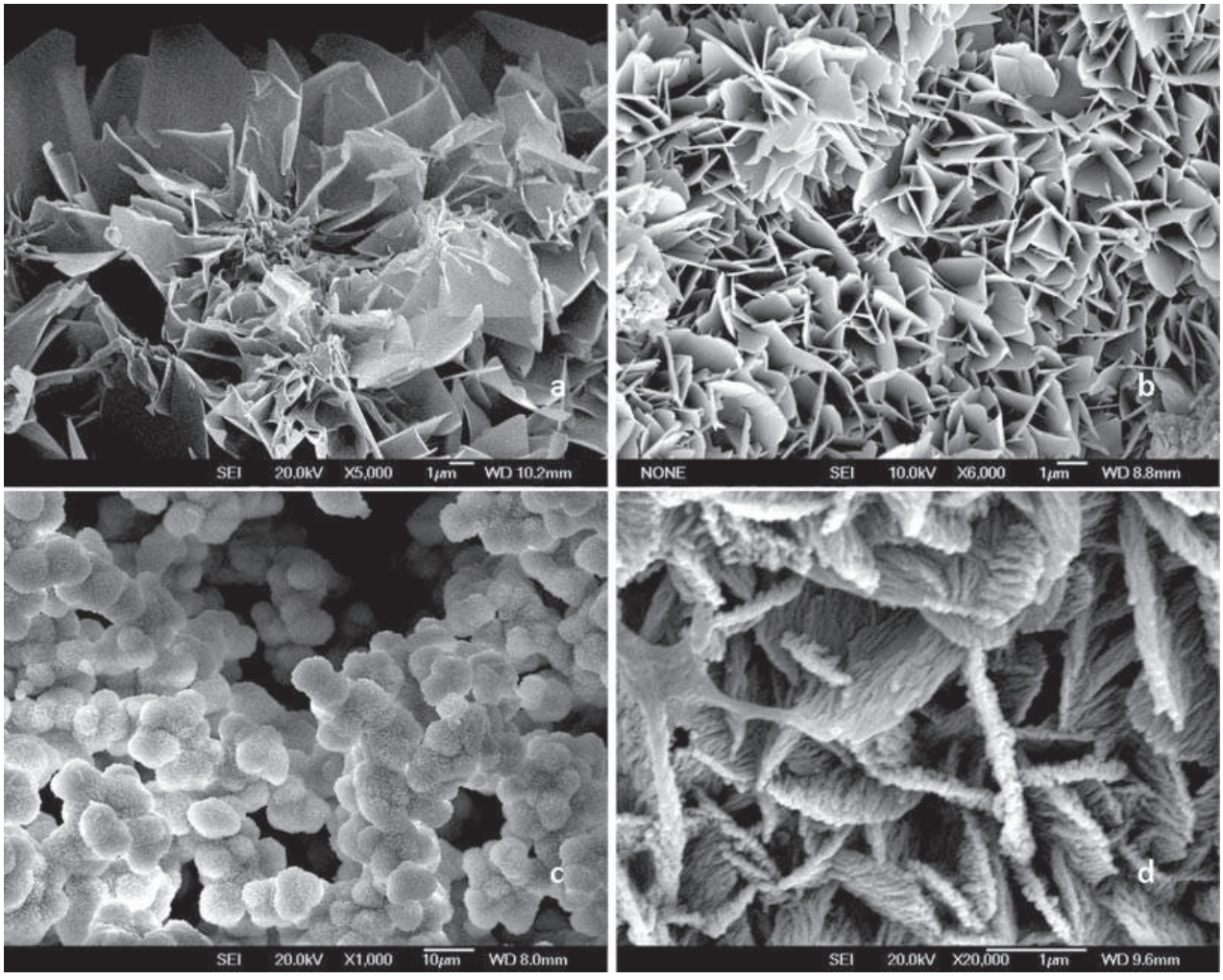


Figure 1 (a) to (c): SEM images showing crystal morphology on Ti discs. (d): SEM in higher magnification showing the detailed surface morphology of the ctystal in (c).

Correlative Light Electron Microscopy for the study of intracellular transport processes.

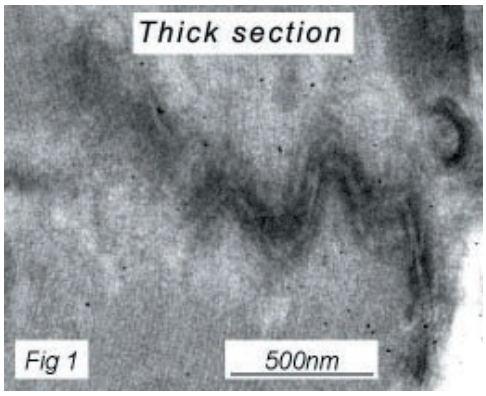
Edward Brown
Department of Biochemistry, School of Medical Sciences, University of Bristol

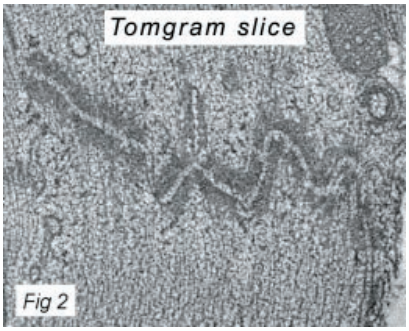
Correlative Light Electron Microscopy (CLEM) aims to combine the best of light and electron microscopy in one experiment. CLEM has certain strengths over the application of both LM and EM techniques separately. Light microscopy (LM) can show the history or sequence of events
between or inside cells and Electron microscopy (EM) provides a much higher resolution image of a particular event and also provides additional spatial information, the so-called reference space. Combining both modalities however generally means making compromises in one or both of the techniques. For CLEM experiments that require a high time resolution together with optimal structural preservation we have developed tools for the integration of High-Pressure Freezing (HPF) in such an experiment. At the moment it is however impossible to combine certain light microscopy techniques, such as for instance Total Internal Reflection (TIRF), with HPF and we have to revert to chemical fixation. We will discuss the different strategies and its advantages and disadvantages.

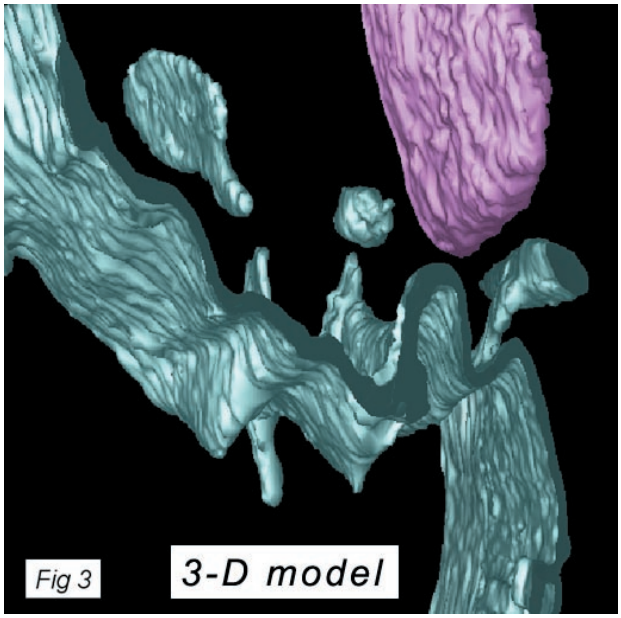
3-Dimensional Visualisation of the Intercalated Disc

Amanda Wilson
Randall Division of Cell and Molecular Biophysics, King's College London

I was awarded this year's Don Claugher Bursary to carry out electron tomography of the intercalated disc, an undulating double membrane that separates adjacent cardiomyocytes. I have captured four double tilt series, reconstructed tomograms, carried out segmentation and surface modeling, and created 3-dimensional computer models. I have combined three adjacent tomograms to make a super montage, and created a unique 3-D model, that shows a 'step' between different portions of the ID. I have used images from a model to augment a scientific poster, and plan to use models, videos and stereograms for further scientific and sci-art projects.







"What's in your barbecue charcoal?! A FE-SEM case-study from Pernambuco State, northeast Brazil"

Dr. Caroline Cartwright
Scientific Research Group, British Museum

Fuelwood and charcoal are of particular value in parts of the world with subsistence economies where fossil fuels are beyond the reach of local people. Charcoal from a wide range of tropical and temperate sources is also sold in the developed world for barbecues. Over 70% of domestic energy consumption in the northeastern states of Brazil comes from such fuelwood and charcoal, and much of it is from native species (Figure 1). The species discussed in this presentation are native to the caatinga (seasonally deciduous forest) of northeast Brazil and are traditionally used there for fuelwood and charcoal production. This work forms part of a collaborative project between the British Museum, the Royal Botanic Gardens Kew and APNE (Associaçao de Plantas do Nordeste), and I would like to mention in particular in this regard, my colleagues from Kew and Brazil respectively, Dr Peter Gasson and Dr Claudia Luizon Dias Leme.

Figure 1 caption: A truck piled high with charcoal made from indigenous Brazilian tree species. Image: Claudia Luizon Dias Leme

Wood retains most of its qualitative features when it is turned to charcoal, but the dimensions and appearance of the cells change in various ways (Figure 2). Wood density, anatomical structure, moisture content, duration and temperature all influence wood behaviour when charred. This presentation illustrates how, using the FE-SEM, it is possible to document in fine detail the qualitative changes that take place in Mimosa wood species when charred artificially and compares them with charcoal produced in a traditional temporary kiln in northeast Brazil.

With the increasing use of native and plantation grown wood for charcoal, there will be greater demand for this type of scientific identification of charcoals to help determine whether the wood has been legitimately or illegally harvested. Archaeologists and palaeontologists are also increasingly in need of reference charcoals to make identifications. These observations are of interest to these users, and those interested in renewable biomass resources, management of woodlands and forests for fuelwood and charcoal, and the ultimate policing of the use of finite fuelwood resources.

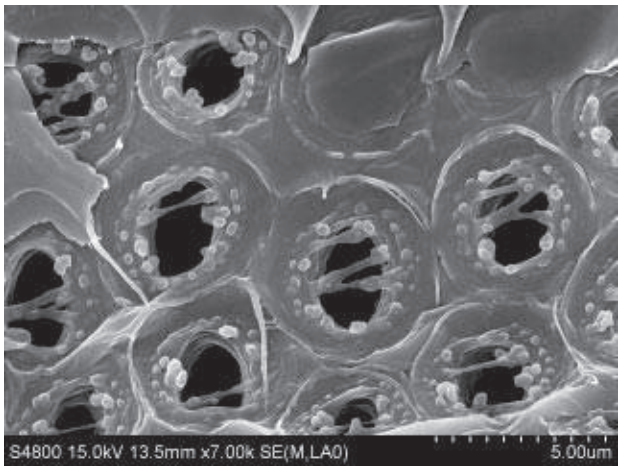


Figure 2 caption: FE-SEM (Hitachi S-4800) image of the radial longitudinal section of Mimosa ophthalmocentra charcoal showing the effect of sustained heat on vestured pit structures

The application of electron microscopy to the investigation of male infertility

Tim Ryder
Charring Cross Hospital

More than one in six couples has difficulty trying to conceive a baby. In more than half of these cases, a male problem is a major contributing factor. The first line of investigation when dealing with an infertile couple is to perform an analysis of the male partner's semen. This examination determines whether the subject's semen profile is within established WHO reference ranges. While this serves to identify that a problem may indeed exist, in many cases it fails to establish exactly what the problem is and, more importantly, how it can be treated. Electron microscopy is a secondary investigation that can help in many of these cases.

There are three main conditions that can benefit from an investigation by electron microscopy. The one that is most often cited is asthenozoospermia (absent or poor sperm motility). In this condition, EM can differentiate between cases where the low sperm motility is the result of an irreversible tail defect caused by a genetic factor, as opposed to reversible acquired tail defects, mitochondrial defects or even sperm death.

Teratozoospermia (excess malformed sperm in the ejaculate) is a condition that has a significant effect on fertility. Here EM allows the identification of acrosomal and chromatin (DNA) defects of the sperm that are not readily detectable by other means.

The third condition where EM can make a contribution is Cytospermia (an excess of non-sperm cells in the ejaculate). EM is considered by some to be the "gold standard" for cell identification and enables the distinction between leucocytes and sperm precursors to be made. A consequence is the significant reduction of the indiscriminate use of antibiotics in these patients.

In an era of developing assisted conception techniques, for example IVF and ICSI, the future role of EM has been questioned. If anything, its value has increased, as it allows patients to make an informed choice between the benefits of choosing assisted conception treatment against the risk of passing on genetic defects to their potential offspring.

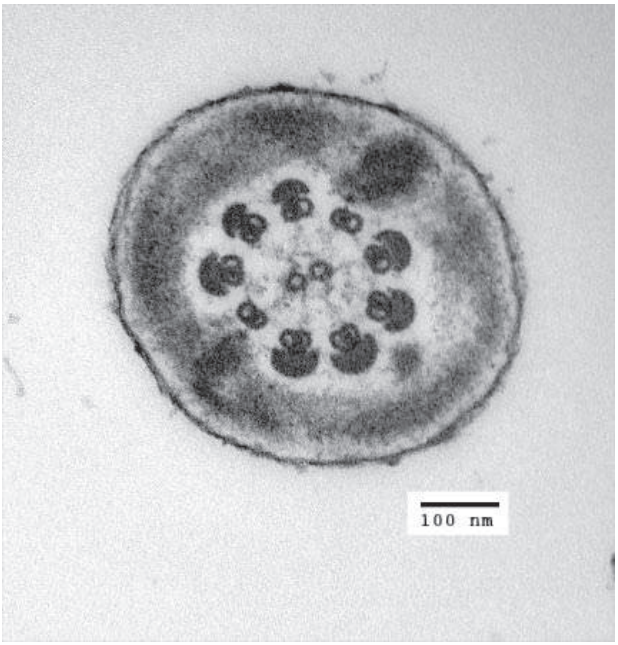


Figure 1: A cross-section of a sperm tail in a case of asthenozoospermia. The condition is the result of Primary Ciliary Dyskinesia (PCD) where the lack of sperm motility is caused by the absence of dynein arms. PCD is an irreversible inherited defect of the sperm axoneme. Bar 100nm.

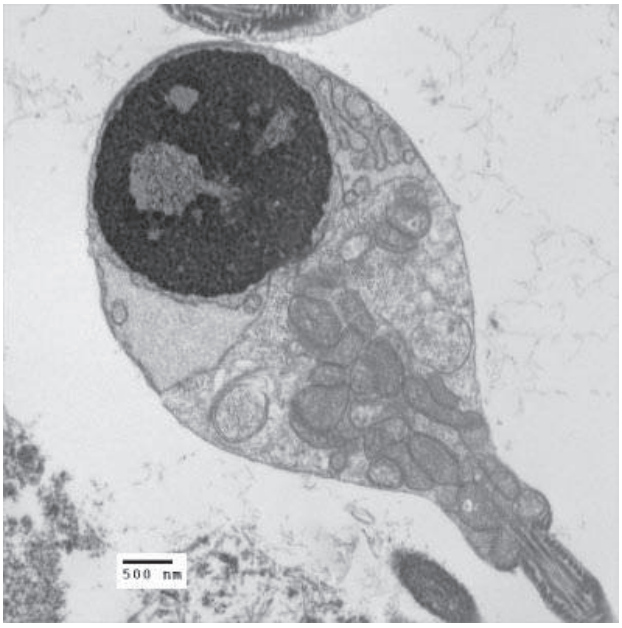


Figure 2: A section through the head and midpiece of a sperm in a case of globozoospermia (round headed defect). The absence of an acrosome makes natural fertilisation impossible and the poorly condensed chromatin reveals the high probability of DNA damage which would negate the value of treatment by an assisted conception technique. Bar 500nm.

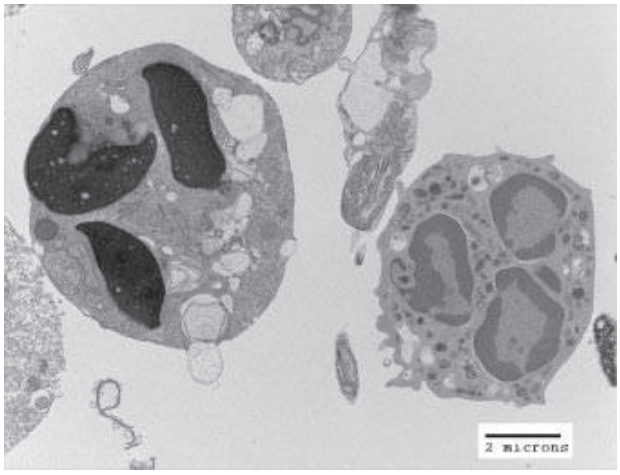


Figure 3: EM allows non-sperm cells to be identified in cases of cytospermia (excess non-sperm cells in the ejaculate). In this case, the neutrophil polymorphonuclear cell on the right is easily distinguished from the aberrant multinucleate spermatid on the left. Examination by conventional light microscopy frequently fails to make this distinction and consequently the inappropriate treatment with antibiotics is given (Bar 2 microns)

3D imaging in optically opaque materials: the structure of mudrocks

Stephen Wilkinson
Imperial College

The description of the structure of a naturally-occurring materials must take into account its three dimensional nature. This is especially true for highly variable materials such as mudrocks. Images of the structure of mudrocks can be described qualitatively, but for engineering purposes it is also important to quantify the structures observed. Image processing techniques provide simple methods for the quantification of 3D structures.

There are two different imaging approaches which are considered three dimensional. Those that create full 3D block models (e.g., x-ray computed tomography, Gatan x-ray ultramicroscope, focused ion beam, transmission electron microscope) and those that create 3D representations of surface topography (e.g., optical microscope (z-stack), interferometer, atomic force microscope, scanning electron microscope (stereo imaging)). When using surface topography techniques several surface orientations have to be observed in order to fully appreciate the structure of materials.

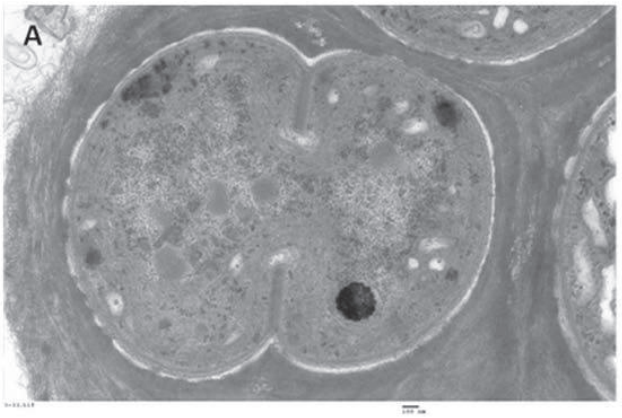
Image processing allows complex structures to be summarised into quantifiable factors. This is often achieved by grouping commonalities, giving an overall idea of the structure of a material. Sometimes the key observation is an irregularity in the material which could not be picked up by automatic image processing. For this reason human interaction and thought is important in unravelling the structures of such complex materials.

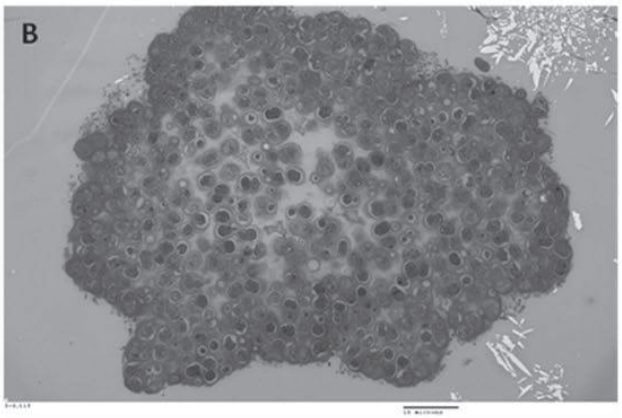
The use of TEM analysis to identify rock-dwelling cyanobacterium resistant to low Earth orbit

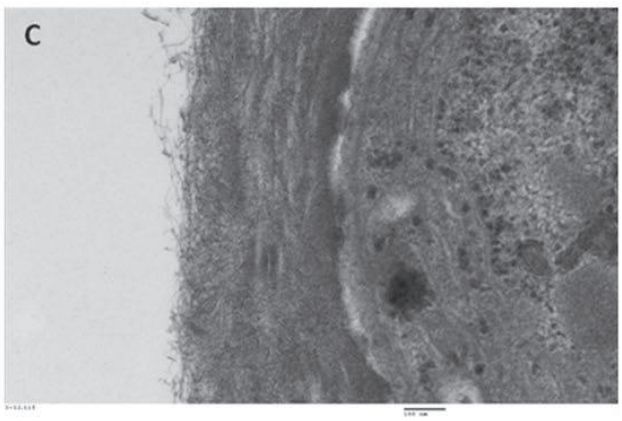
Dr Karen Olsson-Francis
Geomicrobiology Research Group,
Open University

Transmission Electron Microscopy (TEM) was used concomitantly with molecular techniques to identify a cyanobacterium resistant to Low Earth Orbit (LEO). A rock-dwelling community from cliffs in Beer, Devon, UK, was exposed to LEO for 10 days as part of the ESA funded BIOPAN VI mission and returned for analysis. TEM imaging identified the cyanobacterium as a member of the order Chroococcales, as seen in Figure 1A, which was confirmed with phylogenic analysis. Ground based experiments showed that the cyanobacterium, named OU_20, was also able to survive 28 days of exposure to desiccation and Mars simulated conditions, 10 days of exposure to vacuum, and ionization radiation (3 KGy). The ability of OU_20 to survive may be due to the formation of dense colonies, as seen in Figure 1B, or a thick mucilaginous sheath, as seen in Figure 1C. Little is known about the microbial requirements for survival in space. However, TEM analysis has identified physiological characteristics that are potentially key for survival in the adverse conditions on space.

Figure 1: TEM images of the cyanobacterium OU_20 that survived exposure to low Earth orbit







SEMT's first Octogenarian.

This July, Dr. Jill Lewis celebrated her 80th birthday! To mark this very special occasion a celebratory dinner was held at the Royal Institution's new Time and Space restaurant (see photo). It was attended by some of the committee and a previous Chair, Sue Barnes. Jill joined the SEMT in 1982 and became the Honorary Secretary in 1983, a position she held for seventeen years and is currently our Honorary Archivist, still being part of the committee. Jill's enthusiasm for our society gave me the inspiration to become more involved and I gratefully accepted the challenge to become the next elected secretary after Jill, this was ten years ago ! I now appreciate how hard it is to maintain a financially stable society, continue to hold well attended successful meetings and attract new members. I am personally thankful for Jill's support and guidance during my years as secretary and continue to seek Jill's advice on new ventures and always respect her input at committee meetings, so from me a very 'Happy Birthday'.

David McCarthy, Honorary Secretary.

NATURAL HISTORY MUSEUM

Consulting

Analytical facilities

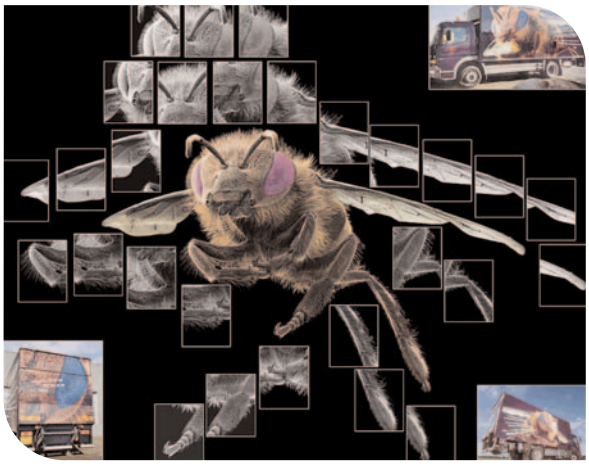
The Natural History Museum offers chemical and structural analysis, and imaging capabilities on a consultancy basis.

The laboratories host a comprehensive range of analytical and imaging facilities staffed by a team of specialists experienced in the preparation, analysis and interpretation of diverse natural specimens.

We can provide a range of services including:

- sample preparation
- optical and electron beam imaging
- chemical analyses
- structural and species determinations of materials
- sourcing of rare or unusual specimens for imaging by electron microscopy

Variable pressure analytical scanning electron microscope



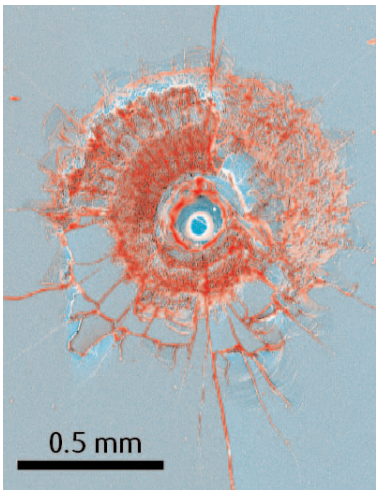
A composite image of a honey bee obtained from 28 overlapping SEM images. The final false-coloured image formed part of a Dutch re-cycling campaign for fridges and other domestic appliances.

What we offer

- preparation of samples for optical and electron imaging, and micro-analysis
- rock cutting, polishing and preparation of rock thin sections
- biological specimen fixation and mounting
- whole sample chemical analyses for the determination of major, trace and ultratrace elements
- micro determinations of trace elements by laser-ablation ICPMS
- laser-scanning confocal microscopy for the production of 3D reflected light or fluorescence images
- cold cathode and scanning electron cathodo-luminescence (CL) imaging for rock and mineral textural analysis
- low-vacuum scanning electron microscopy and micro-analysis of unprepared samples
- high resolution SEM imaging of prepared samples
- false-colour processing of images
- single mineral U/Pb age determinations by LA-ICPMS
- fully quantitative electron probe micro-analysis and element mapping
- qualitative microprobe analysis of uncoated samples
- automated SEM-EDX image and elemental analysis, including feature characterization
- transmitted electron microscopy of biological ultra-thin sections
- x-ray diffraction (XRD) to identify, characterize and quantify mineral phases in mixtures
- infrared spectroscopy for characterization of material in transmission and reflectance modes

Some recent contracts

Monitoring of atmospheric emissions from the Karabash copper smelter. The samples were later characterized using imaging and analytical techniques



Micrometeoroid impact crater on a solar cell from the Hubble Space Telescope

Position sensitive detector used in the rapid characterization and phase quantification of materials by X-ray diffraction

Environmental assessment in the South Urals, Russia
for European Commission
Instrumental- and bio-monitoring methodologies were used to assess environmental impact of mining-related activities and to suggest low cost alternatives for remediation and best practice for future extraction activities.

Palaeo-environmental study
for James Cook University, Australia
A geochemical and isotopic analysis of peat material was performed as part of a palaeo-environmental study in Northern Australia.

Geochronology of minerals
for NHM, Dresden, Germany
Laser-ablation ICPMS was applied to date zircons and other minerals from a variety of geological environments for the mining and exploration industries.

Space debris analysis
for the European Space Agency
A study of the micro-impact damage by natural and man-made particles was undertaken in sections of solar panels from the Hubble Space Telescope using high-resolution imaging and micro-analysis.

Environmental assessment study
for London Borough of Bromley
Airborne particulates collected on filters from various road-side localities were fully characterized in terms of their elemental composition, size and shape. These data could be used to assess possible impacts on human health.

Mineralogy of reservoir rocks
For Cambridge Carbonates Ltd
A petrographic and mineral chemistry study using optical and electron beam techniques was undertaken to assess rock porosity for the oil industry.

Phase analysis
for the London Crossrail Project
Quantitative phase analysis of sands and muds from tunnelling sites have been undertaken by X-ray diffraction.

Characterization and verification of artefacts
for Bonhams Auctioneers
Several contracts have been undertaken with auctioneers to verify the natural components present in a variety of artefacts. A combination of imaging, micro-analysis and structural information was used for these studies. We are able to test material non-destructively, and without damage to the sample.

Documentary and film making
for BBC, Channel 4
Several documentary makers have used our analytical facilities, where high resolution images and analysis of materials were required.

For further information contact

Department of Mineralogy
Natural History Museum
Cromwell Road
London
SW7 5BD

Terry Williams
Telephone +44 (0) 207 942 5663
Facsimile +44 (0) 207 942 5811
Email t.williams@nhm.ac.uk
www.nhm.ac.uk/science

Christine Ung (General enquiries)
Telephone +44 (0) 207 942 5816
Facsimile +44 (0) 207 942 5841
Email c.ung@nhm.ac.uk

ds1680