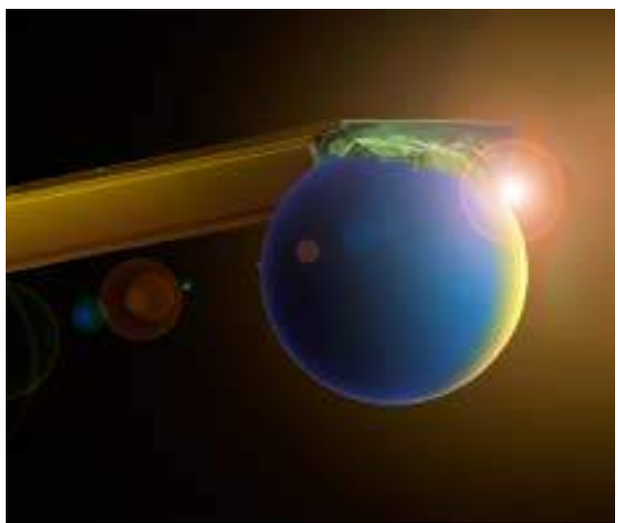
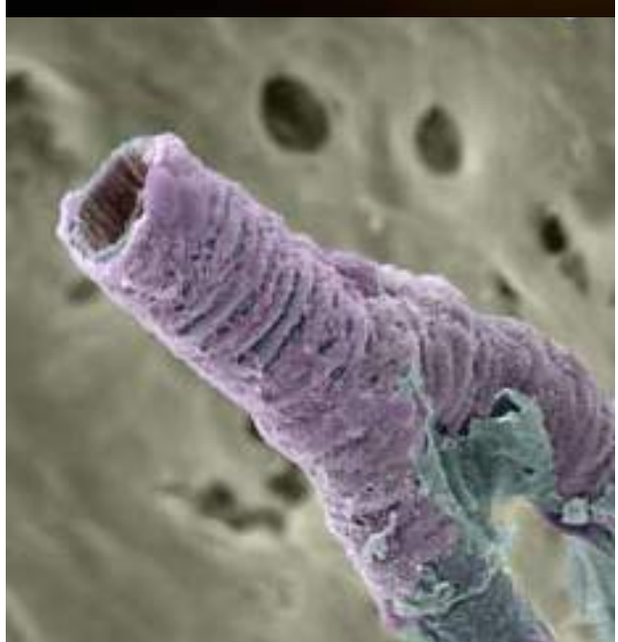
Keeping you informed of the latest technology & techniques

Society of Electron Microscope Technology

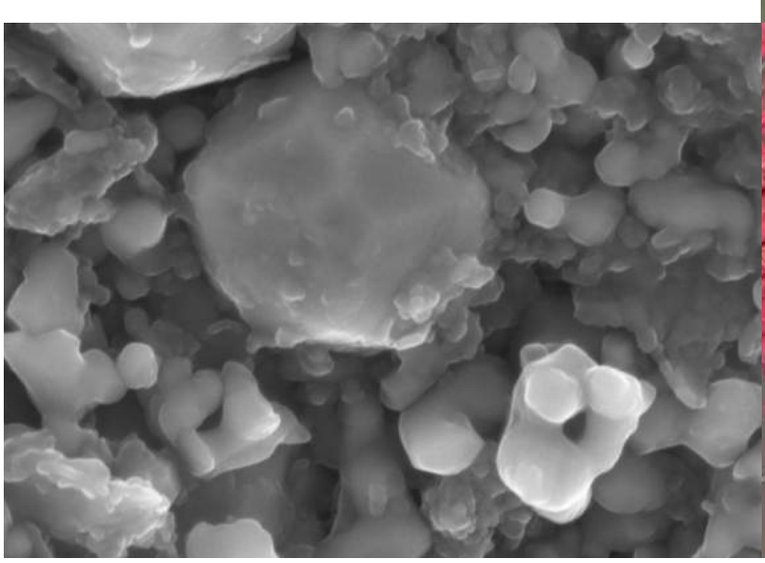


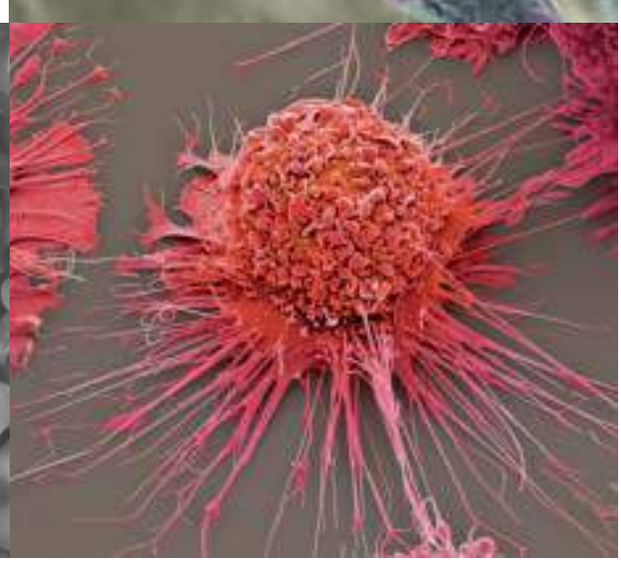


SEMT
One Day Meeting

Wednesday 18th December 2013

at
UCL School of Pharmacy





Society of Electron Microscope Technology

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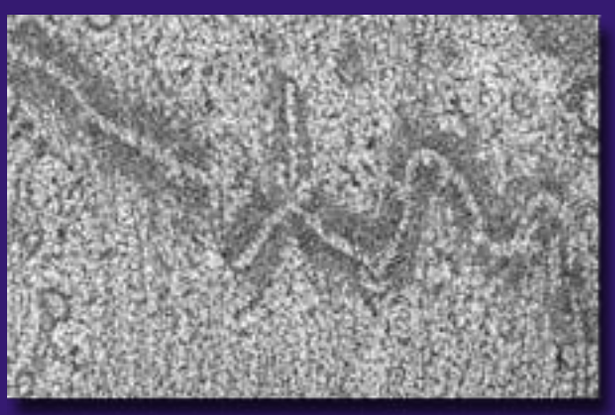

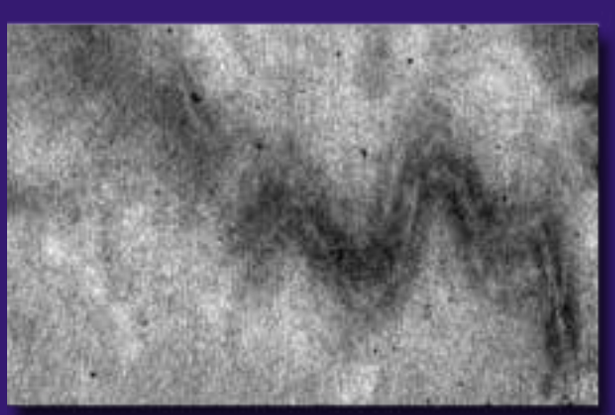

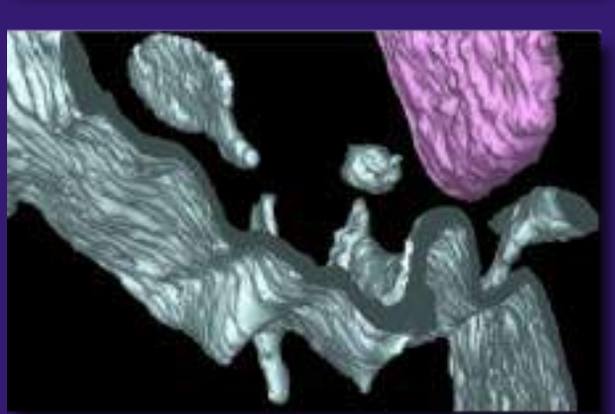

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Acknowledgments

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Future Programme
One day Meeting, 17th December 2014

09.15 Registration, Tea & Coffee, Trade Exhibition

09.55 Introduction: Chair, **Heather Davies**

10.00 **Children or Career? What's the decision?**
Pia Østergaard - Daphne Jackson Trust

10.35 **Scratching the surface – Applications of micro & nano imaging in the medical devices industry**
Paul Gunning - Smith & Nephew Research Centre, York

11.10 Tea & Coffee, Trade Exhibition.

11.30 **The role of ultrastructural examination in kidney allograft rejection – research and diagnosis**
Candice Roufosse – Imperial College London, NHS Trust.

12.05 ***RMS Beginners Competition***

1. **Small gold nanoparticles: potential carriers of therapeutics across the blood-brain barrier**
   Radka Gromnicova - The Open University
2. ***Origin of pristine matrix grains in unequilibrated chondritic meteorites***
   Epifanio Vaccaro - The Natural History Museum
3. ***The Applications of Micro-CT Within Meteoritics***
   Natasha Vasiliki Almeida - The Natural History Museum
4. **Down the Microscope: The successes and limitations of Micro-CT and developing methodologies for comparative CT and confocal microscopy studies**
   Rebecca Summerfield - The Natural History Museum

13.05 Buffet Lunch & Trade Exhibition

14.30 **The role of microscopy in the development of toothpastes**
Jonathan Earl - GSK Oral Healthcare

15.05 **DIRECT DETECTION - The next big thing for TEM cameras?**
Neil Wilkinson - Gatan UK Ltd.

15.40 Tea & Coffee.

16.00 **The pathogenesis of Alzheimer's Disease**
Alan Morris - Southampton General Hospital

16.35 **The importance of the wider use of scientific imaging**
Steve Gschmeissner - theworldcloseup.com

17.10 AGM - Wine Reception – *Sponsored by Carl Zeiss Ltd.*

18.30 Conference Dinner

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Children or Career? What's the decision?

Dr. Pia Østergaard
Senior Fellowship advisor, Daphne Jackson Trust & Lecturer at the Human Genetics Research Centre, St George's University of London

The Daphne Jackson Trust (http://www.daphnejackson.org/) provides a returners scheme helping those with a background in science, engineering and technology to return after a career break. It is an independent charity which offers flexible, part-time, paid fellowships to researchers who have taken a career break of two or more years for family, caring or health reasons. The most important part of the programme is the provision of training and updating of skills via a research project.

I will tell you about my own experience as a Daphne Jackson Fellow and how I have returned to science. I will give examples on how I have managed my work-life balance, which last year even led to an award. Besides working for St George's University of London as a Lecturer in Human Genetics, I also work for the Daphne Jackson Trust as a fellowship advisor, and I assist the Trust in a variety of activities related to the fellowship selection process.

Scratching the surface – Applications of micro & nano imaging in the medical devices industry

Paul A. Gunning
Smith & Nephew Research Centre, York Science Park, Heslington, York, YO42 2NB, UK.
paul.gunning@smith-nephew.com

Osteoarthritis affects more than 70% of adults between 55 and 78 years of age in the USA alone; up to 38% of these individuals are likely to require replacement joints (27% of population). The percentage of the population, young and old, requiring advanced wound care is likely to be substantially higher, with older patients more likely to need longer-term wound care. With an ageing demographic and rising healthcare costs in most of the developed (and some of the developing) world, improving the effectiveness and affordability of medical devices is of ever increasing importance.

The development of successful new medical devices requires input from most scientific disciplines and microscopy is often a pivotal method in understanding material-material and biological tissue-material interactions through 'direct' visualisation. This talk aims to provide an overview of the applications of micro and nano imaging methods for new product development, quality assurance/regulatory and pseudo-forensic troubleshooting activities. Each application area is illustrated using several real-world examples including studies of active pharmaceutical loaded micro-spheres (Fig. 1), physico-chemical and bio-compatibility analysis of a nano-structured titanium bone in-growth material (Fig. 2) and examples of industrial troubleshooting/contaminant analysis. Future trends in materials and analytical approaches pertinent to the medical devices industry are also discussed.

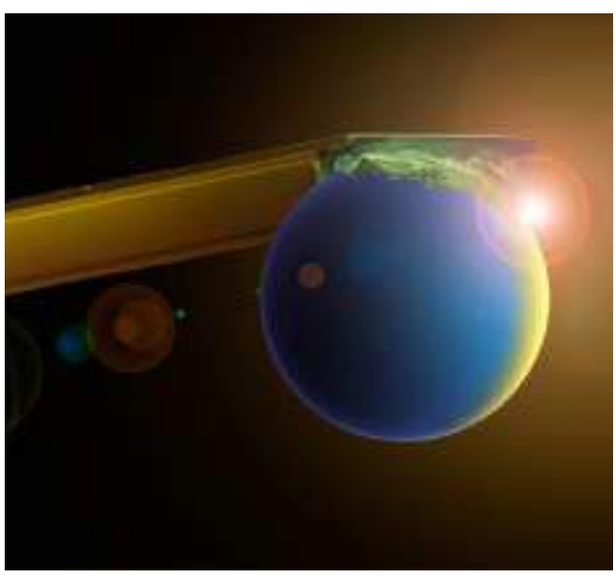

Fig.1: Micro-sphere attached to AFM tip

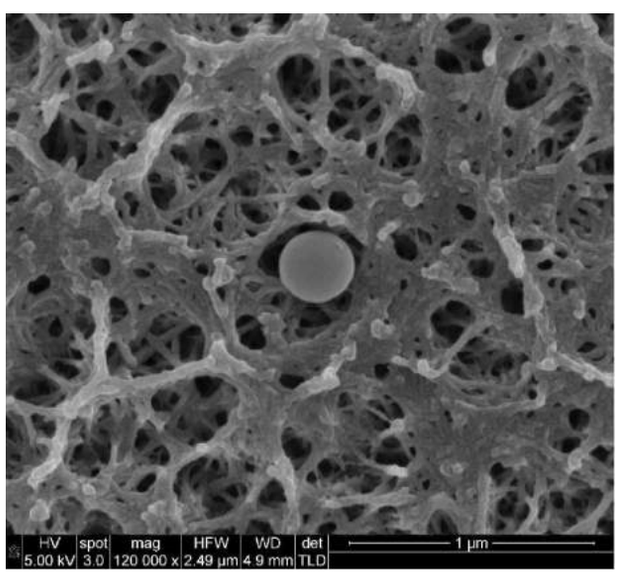

Fig.2: Matrix vesicle on titanium surface

The role of ultrastructural examination in kidney allograft rejection – research and diagnosis

Candice Roufosse
Imperial College London, NHS Trust.

Electron microscopy is considered a key component in the histological examination of native kidney biopsies. Its role in the evaluation of the transplanted kidney is more controversial. In addition to its role in investigating recurrent glomerular disease, it is also used to evaluate glomerular changes and peritubular capillary basement membrane multilayering (PTCBML), in order to help establish a diagnosis of chronic rejection. More recently, subtle changes in the renal microcirculation (microcirculation = glomerular and peritubular capillaries), which are invisible on light microscopy, have been noted on ultrastructural examination of patients who either go on to develop chronic rejection, or who are at high risk of developing it. This has helped to establish renal microcirculation injury as a central event in the pathogenesis of chronic rejection. Currently, more emphasis is being put on early diagnosis of chronic rejection, at a stage where it may be more amenable to treatment - detailed examination of the renal microcirculation using EM is a contender in the various competing diagnostic modalities being considered to increase early detection of this condition.

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Ivanyi B, Fahmy H, Brown H, et al. Hum Pathol 2000;31: 1129-1138

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Wavamunno MD, O'Connell PJ, Vitalone M et al. Am J Transplant 2007;7: 2757-2768.

Early Ultrastructural Changes in Renal Allografts: Correlation With Antibody-Mediated Rejection and Transplant Glomerulopathy.
Haas M, Mirocha J Am J Transplant 2011

¬Peritubular capillary basement membrane multilayering on electron microscopy: a useful marker of early chronic antibody-mediated damage
Roufosse C, Shore I, Moss J et al. Transplantation 2012;94(3):269-274

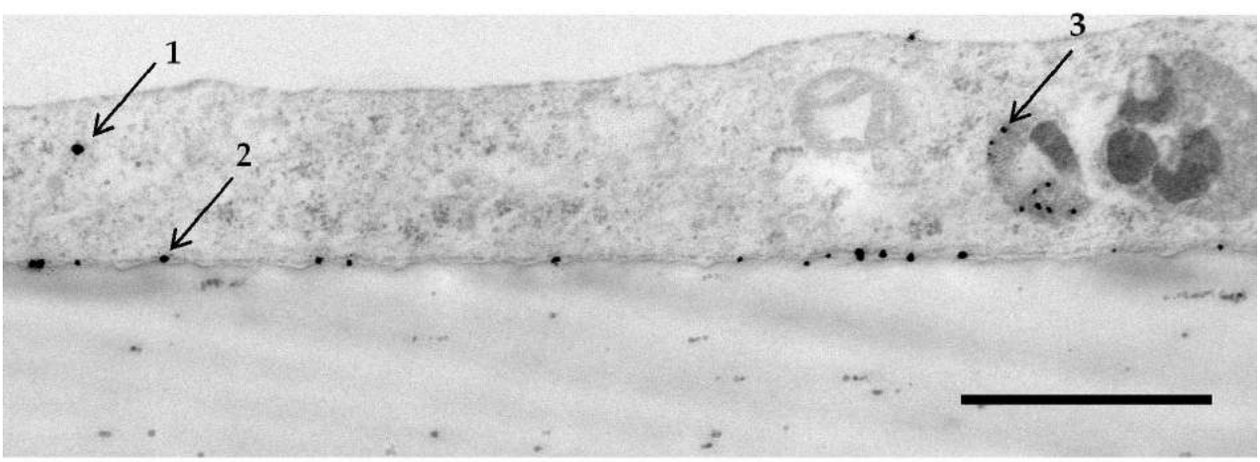
RMS Beginner's Competition

Small gold nanoparticles: potential carriers of therapeutics across the blood-brain barrier

1.

Radka Gromnicova
The Open University, UK

The blood-brain barrier prevents up to 95 % of drugs from entering the brain which makes treatment of brain disorders challenging. The emerging field of nanomedicine can offer help in the form of nanoparticles designed to cross the blood-brain barrier and deliver a cargo. But the first step is to identify an appropriate carrier. We investigated 4 nm gold nanoparticles, which are covalently coated with glucose. These nanoparticles entered brain endothelial cells and were able to exit the cell on the basal side, moreover, they appeared to enter the cell cytosol directly rather than via vesicles. The mechanism of entry of nanoparticles appears to be based on the biophysical properties of the plasma membrane such as its fluidity. Next, we compared uptake into and across vascular endothelia from different tissues; brain endothelial uptake was the most effective. Lastly, we used our 3D in vitro co-culture model where endothelium is cultured on top of a collagen gel containing astrocytes and in this case, the nanoparticles were observed both in the endothelium and astrocytes. Therefore, this type of nanoparticle appears to have the potential to carry a therapeutic cargo across the blood-brain barrier.



Transmission electron micrograph of a brain endothelial cell (cell line hCMEC/D3) containing gold nanoparticles (arrows). The cell is grown on a plastic porous membrane. The gold nanoparticles, which are 4 nm glucose-coated, are observed inside the cytosol (1), at the bottom of the cell (2) and in endosomes/granules (3). Scale bar = 1 micron.

Origin of pristine matrix grains in unequilibrated chondritic meteorites

2. **Epifanio Vaccaro**
Mineral and Planetary Sciences,
The Natural History Museum, London.

Carbonaceous chondrites are among the most chemically pristine planetary materials available for study. These meteorites provide important constraints on the diversity of processes that occurred in the early solar system.
In particular the CO3 and CR chondrites are among the most primitive meteorites which have been minimally affected by low-temperature aqueous alteration. The mineralogical and chemical characteristics of the fine-grained matrix (<3μm) of primitive carbonaceous chondrites have been investigated in detail by scanning electron microscopy (SEM) and analytical transmission electron microscopy (ATEM).Generally, the fine-grained matrix represents a highly unequilibrated assemblage of an amorphous material.
The aim of this project is to characterise the mineralogical diversity of primitive matrix (morphology, grain size and mineralogy) of pristine chondritic meteorites in 2D and 3D to learn about its origins and thermal and chemical evolution; we also aim to investigate the early stages of aqueous alteration and assess to which extent this sample have been altered.
The Natural History Museum has recently developed instrumentation suited to the investigation of sub-micron sized materials such as matrix, using Field Emission Gun scanning electron microscopy (FEG-SEM).Ultra High resolution secondary and backscattered electron imagines, of the selected matrix regions, were taken utilizing FEG SEM endowed with the new in-lens detector technology.
We are undertaking a comparative petrologic study of different chondrite groups, and micron-sized foils are being cut from areas of interest identified by FEG-SEM, at the Open University in Milton Keynes, using the focused ion beam (FIB) technique, the structure of these samples will then be investigated using Nano-CT. After this initial characterisation work, we will expand this work to study of isotopic characterisation by Nano SIMS at the Open University, in Milton Keynes, UK.

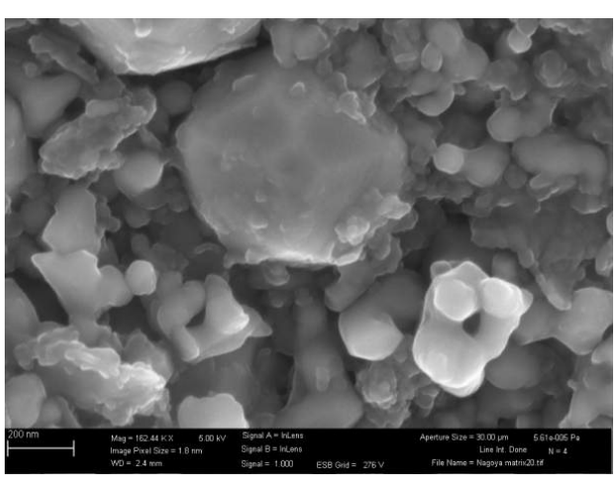


Fig 1 Matrix of altered carbonaceous chondrite Nagoya characterised by framboidal magnetite crystals.

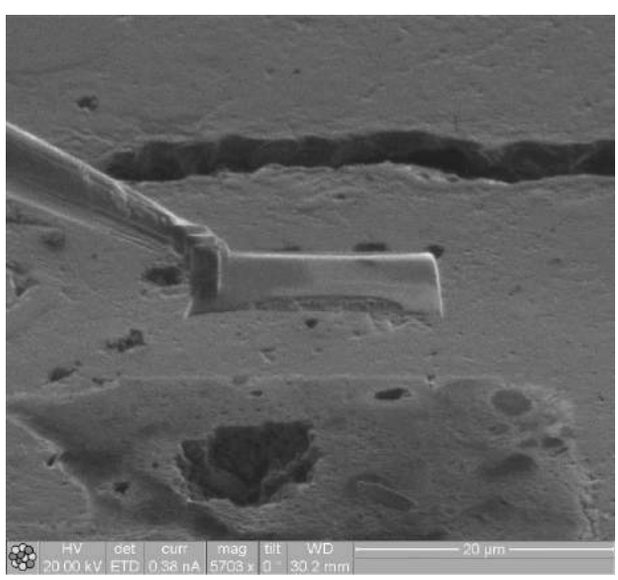


Fig 2 Foils of Matrix are being cut from areas of interest using the focused ion beam (FIB) technique.

RMS Beginner's Competition

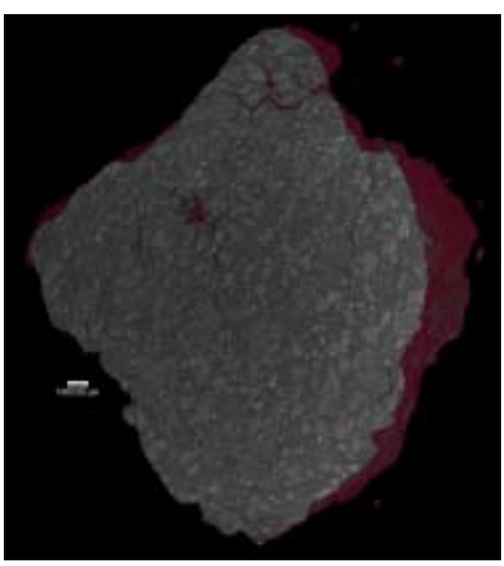
The Applications of Micro-CT Within Meteoritics

3.

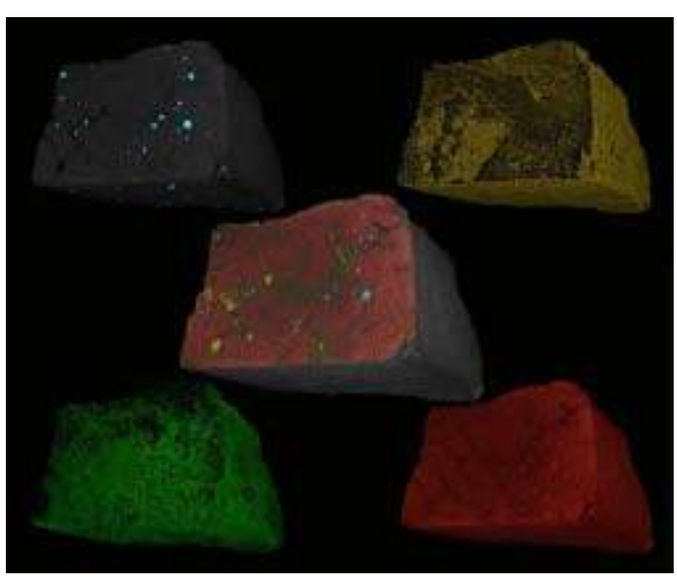
Natasha Vasiliki Almeida
Department of Earth Sciences
The Natural History Museum

Due to the rare and precious nature of extraterrestrial material, micro-computed tomography offers a crucial non-destructive and non-invasive method for investigating the mineralogical and textural features of such samples. Meteorites can afford insight into the characteristics and processes of the early Solar System. Martian meteorites, specifically, provide a window into volcanism and water both on the surface and subsurface.

This project exploits the Nikon HMXST 225 Micro-CT system at the Natural History Museum, London, in order to elucidate the three-dimensional properties of extraterrestrial samples, in contrast to more conventional two-dimensional analyses, using traditional sectioning. Our systematic study and documentation of the NHM meteorite collection will be introduced. This will include examples of how the technique has thus far been used, complementary scanning electron microscopy and intended studies for the future.



CT slice of the Tissint meteorite, with the air/rock interface coloured in magenta to highlight the voids within the sample.



Composite of CT images to show the different phases within a sample of the Martian meteorite, Chassigny.

Down the Microscope: The successes and limitations of Micro-CT and developing methodologies for comparative CT and confocal microscopy studies

4.

Rebecca Summerfield
Micro-CT lab, Imaging and Analysis Centre, Science Facilities, The Natural History Museum.

Micro and nano-CT are important non-destructive techniques for creating visual datasets that elucidate textural and structural information from a diverse range of materials, enabling systematic and taxonomic studies of precious biological and geological specimens. The Natural History Museum's Imaging and Analysis Centre is a world leader in creating novel and widely applicable CT methods. As such, it collaborates with academics from around the globe on a diverse range of projects from the prey of deep sea angler fish (Fig. 1) to examining mining cores for industry. This talk will utilise examples from the most recent studies to explain the importance and limitations of these techniques.

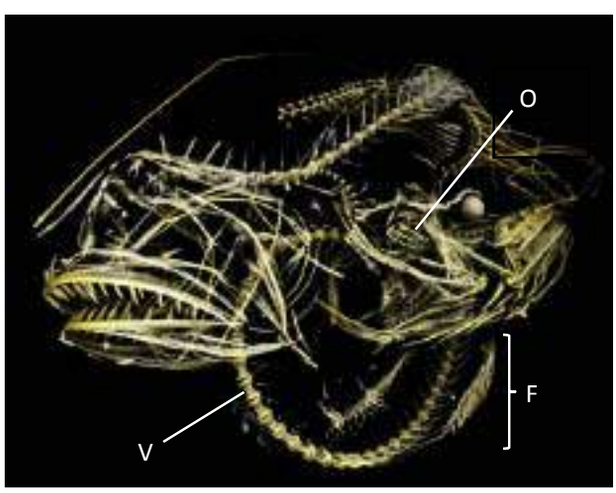


Figure 1. Extracted skeletons of Caulophryne pelagica[1] (a deep sea anglerfish) and Roulenia atrrita its prey[1]. Features used for identification: number of vertebrae (V), number of fin bones (F) and the otoliths shape (O).

The restricted range of densities in biological tissues reduces contrast in, and thus complicates the interpretation of, 3D datasets. Stains have been shown to improve contrast in a variety of vertebrates and a limited range of invertebrates e.g. polychaetes (see Fig. 2-3) and terrestrial arthropods. For micro-CT specimens are usually scanned in ethanol. However, for nano-CT, which is an SEM-hosted technique, using the Gatan XuM system in our FEI Quanta 650 SEM the specimens must be dried to withstand exposure to the vacuum. As a result, the stains we use must be consistent throughout the dehydration and drying preparations. This study compares the effectiveness of three commonly used micro-CT stains (osmium textroxide, phosphotungstic acid and iodine) for SEM-hosted nano-CT and compares them with data obtained via confocal microscopy, which offers similar resolution, but does not require the samples to be stained or dehydrated.

Figure 2. Longitudinal section of a deep sea polychaete worm[2] stained with PTA and scanned with a Skyscan 1172 micro-CT system, Hellenic Centre for Marine Research, Greece.

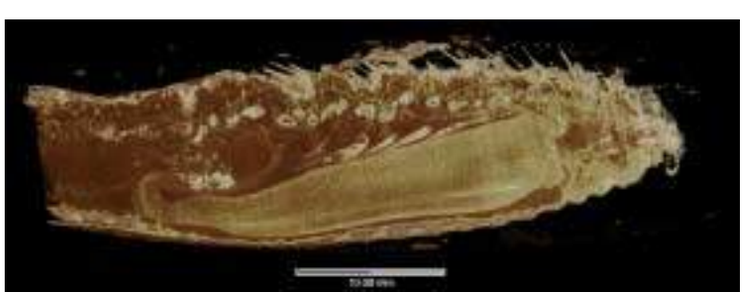


Figure 3. Longitudinal section of an unstained deep sea polychaete worm[2], Aphrodita sp., specimen scanned with a Nikon HMXST 225 micro-CT system, The Natural History Museum, London.

[1]James Maclaine, Life Sciences, The Natural History Museum. [2]Dan Sykes, Facilities, The Natural History Museum.

The role of microscopy in the development of toothpastes

Jonathan Earl
Principal Scientist, Sensitivity and Acid Erosion R&D, GlaxoSmithKline Consumer Healthcare, Weybridge, Surrey, UK.

Dentine hypersensitivity is an increasing problem, characterised by short, sharp pain arising from exposed dentine. GSK has recently introduced a new daily desensitising toothpaste containing the active ingredient NovaMin®, specifically designed for dentine hypersensitivity patients.

In this talk, the role that electron microscopy has played in the development and communication of this new product will be discussed. Including the use of SEM, ESEM, TEM, FIB SEM, STEM, EDS, SAED and in-situ SEM nano indentation

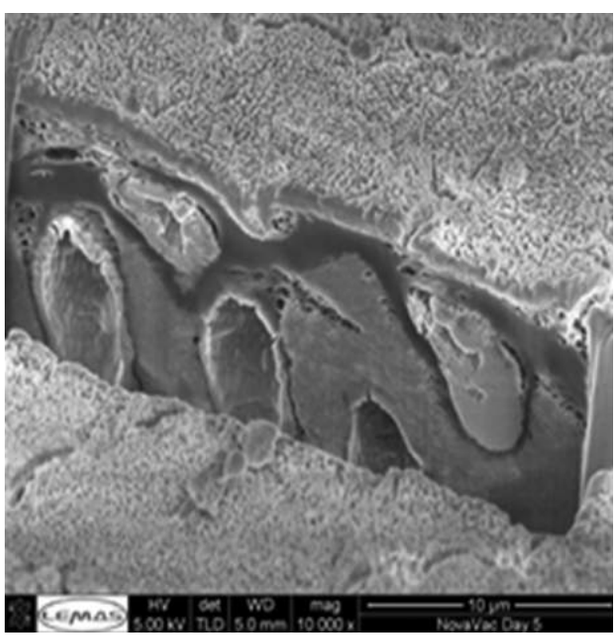


Figure 1: FIB SEM prepared cross section showing surface layer and occluded dentine tubules

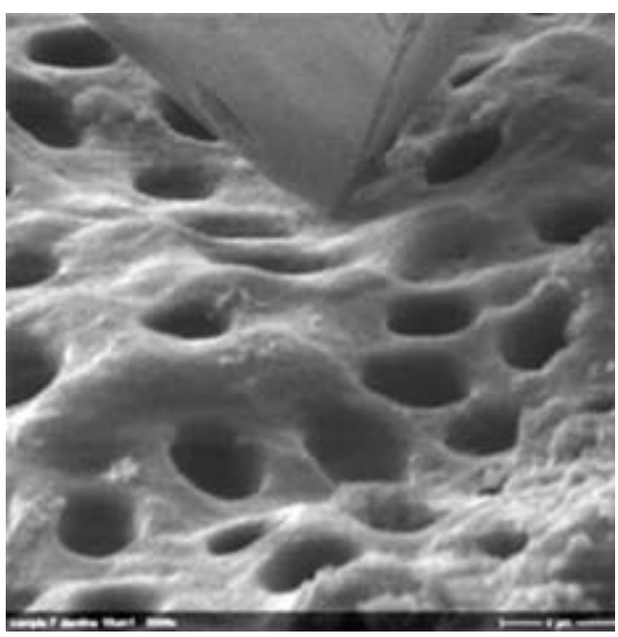


Figure 2: SEM image of a dentine hardness measurement

DIRECT DETECTION - The next big thing for TEM cameras?

Neil Wilkinson
Gatan UK Ltd.

Introduction – the development of modern CCD cameras for TEM

CCD cameras have been in use for TEM image capture since 1990, when the first commercial systems became available. These cameras were aided by developments in Astronomy where large funding was available for the development of big new telescopes, leading in turn to TEM Camera designs utilising the fast developing Charge Coupled Detector – CCD. Since their first development at the Bell Labs in 1969, CCD's began to be used in a wide range of imaging and spectroscopy applications. Using this technology and high quality fibre optic blocks, TEM cameras were developed. Although the early 512 x 1024 pixel Fibre Optically coupled CCD cameras were not large by modern standards, they were very high resolution and began to offer a high quality alternative to film. In addition to digital imaging, the Computer systems could analyse images on-line, and feed back the information to the TEM, to optimise, focus and align.

CCD based cameras are now available with up to 10k x 10k pixels with single pixel resolution and high detection efficiency.

Fibre optically coupled CCD cameras occupy the high quality end of the market. The lower cost Lens coupled CCD cameras offer an alternative where best image resolution and sensitivity are not required.
The CCD camera has become a standard feature on almost all TEM's for all materials and almost all Life Science applications.

However, photographic film is both very sensitive and very high resolution – higher in both respects than all but the most expensive CCD cameras - at a cost in excess of £500K!! For this reason, some of the most demanding applications have, until recently, continued to use film.

Low Dose Cryo TEM – the most difficult image capture problem.

In Cryo Electron Microscopy, the sample is dispersed in a liquid, placed on a Holey Film and plunged into Liquid Ethane. In this demanding sector of TEM, the sample must at all times be kept cold with a special Liquid Nitrogen cooled holder. The electron dose must be limited to one short exposure, during which specimen damage occurs with no possibility for a second look. The dose rate typically is in the range of 10 – 20 e/Å2. The images are extremely noisy and have very low count rate/intensity as the exposure conditions necessary are very extreme.
The cryo community has been the target for many specialist developments to gain improvements, to allow higher quality and resolution 3D reconstructions and better cryo tomograms. These developments include Phase Plates, Electron decelerators and other complicated specialist cameras. Direct detection cameras are among these developments.

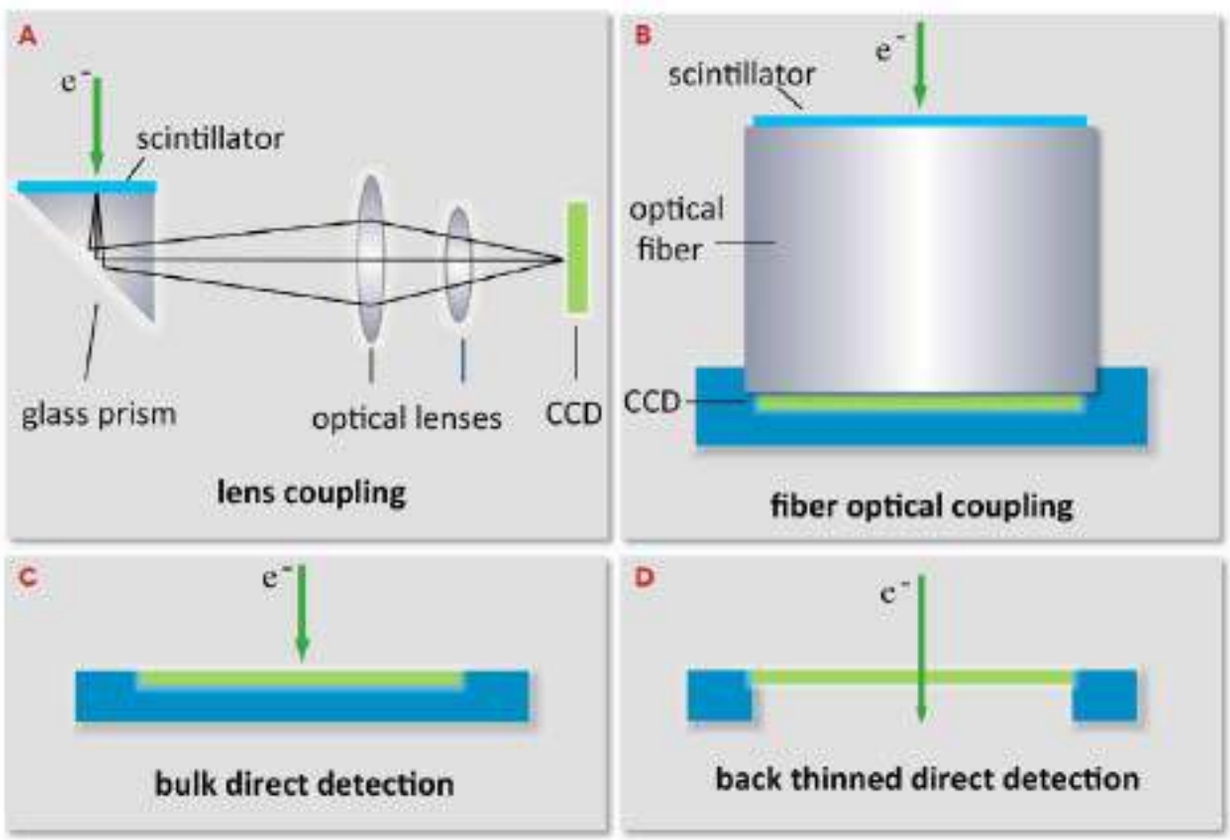
Direct Detection – new detectors prove higher sensitivity and very high resolution.

The Direct Detection camera has proved to be the answer. By removing the lenses, scintillator and fibre optics, electrons can be detected directly onto a Silicon device. In principle, this would gain both better resolution and sensitivity. However, High energy electrons prove extremely damaging to CCD's and their life is very short, with degradation rendering them un-usable very quickly.

From Monte Carlo calculations of electron trajectories in Silicon, it can be calculated how much energy is deposited into the detector and scattering in the detector. Thinning the detector means less scattering and damage will occur, due to the reduction in the electron interaction volume. Such "Back-thinned" detectors have proved to be the way forward for Direct Detection cameras.

continued.....

Neil Wilkinson
Gatan UK Ltd.



Fast Readout and Electron Counting – the way to go for Cryo and In Situ.

By reading out the camera very fast, and summing the counts externally, images can be built up in Firmware/software. Fast readout also means Dose Fractionation becomes possible, with correction for Image drift, and removal of frames where the sample can be seen to be damaged. Some cameras now read out at speeds up to 1600 Frames per second. This allows them to be used, not just in Cryo, but for In-Situ TEM applications.

Direct Detection cameras are already in use in many cryo labs for Single particle and Cryo Tomography applications. Several labs, specialising in In-Situ applications have begun to produce remarkable images from Direct Detection cameras.

The Future?

There is little doubt that in the Cryo and In Situ applications areas, Direct Detection is the way forward. Because these detector systems are so very specialist, it may take some time to filter down to normal TEM usage.

Understanding the role of Apolipoprotein E in Alzheimer's disease

Alan W J Morris, Cheryl Hawkes, James Nicoll, Roxana O Carare
Faculty of Medicine, University of Southampton, UK

Dementia is an increasing problem in our ageing population. A new diagnosis of dementia is made every 4 seconds and it is estimated that there will be more than 125 million people worldwide with dementia by 2040. Alzheimer's disease (AD) is the commonest form of dementia with 496,000 people in the United Kingdom currently affected. In AD there is a failure of elimination of amyloid-β (Aβ) and other waste products from the brain. These proteins accumulate in blood vessels that supply the brain, preventing efficient blood flow and subsequently killing brain cells. Our group has demonstrated that normally Aβ is removed from the brain along Basement Membranes (BMs) which are networks of proteins 100-150nm in thickness present deep within the walls of blood vessels. Apolipoproteins (Apo) are proteins that carry cholesterol between cells. People who have the ApoE4 protein are more likely to develop AD but the reason for this susceptibility is unknown. Our working hypothesis is that with increasing age and in the presence of ApoE4 drainage of Aβ along BMs is disrupted. Following an experimental model of intracerebral injections, here we demonstrate that the pattern of drainage of Aβ from the brain is smooth along basement membranes when Aβ is injected alone, but it forms a halo around the blood vessels in the parenchyma following pre-incubation of Aβ with ApoE4. These findings provide new information not only about why apoE4 is a risk factor for AD, but may also lead to new strategies which focus of maintaining healthy blood vessels in the prevention of AD. This work may also identify novel therapeutic strategies to aid clearance of Aβ.

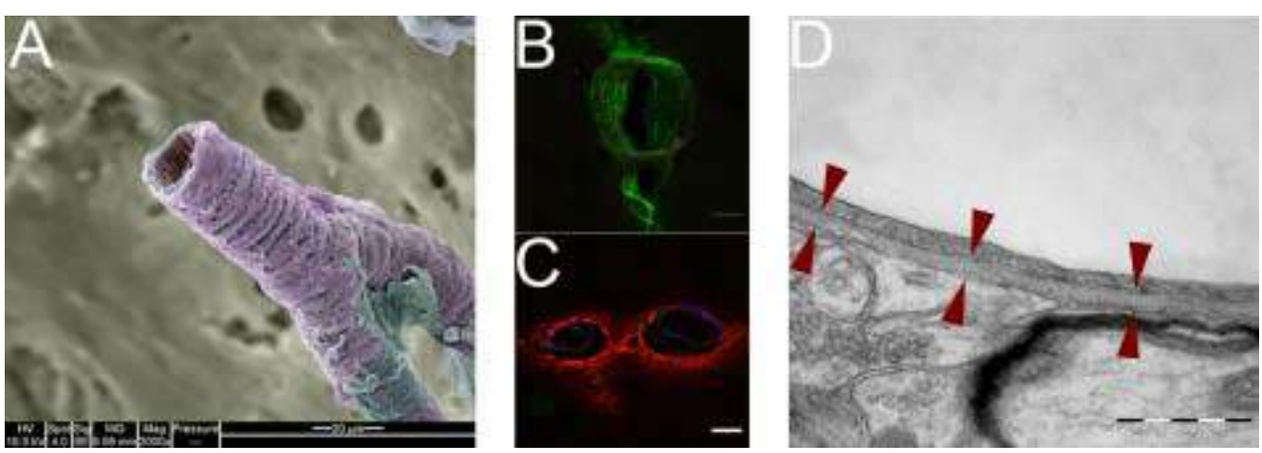
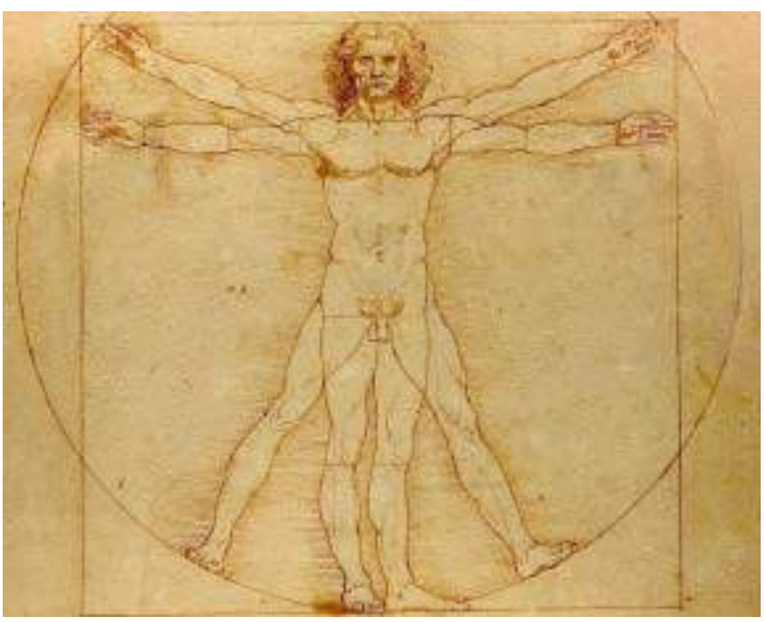


Figure 1
A; False colour SEM image of a Wistar rat cerebral artery.
B; Confocal image showing the drainage of Aβ (green) along a cerebral artery in the brain of a mouse.
C; Confocal image showing the pattern of drainage of Abeta (red), following incubation with ApoE4, along a cerebral artery in the brain of a mouse. Smooth Muscle Actin (SMA) is blue and laminin is green. D; TEM micrograph of Wistar rat cerebral capillary. The BM is the electron dense region between arrow heads.

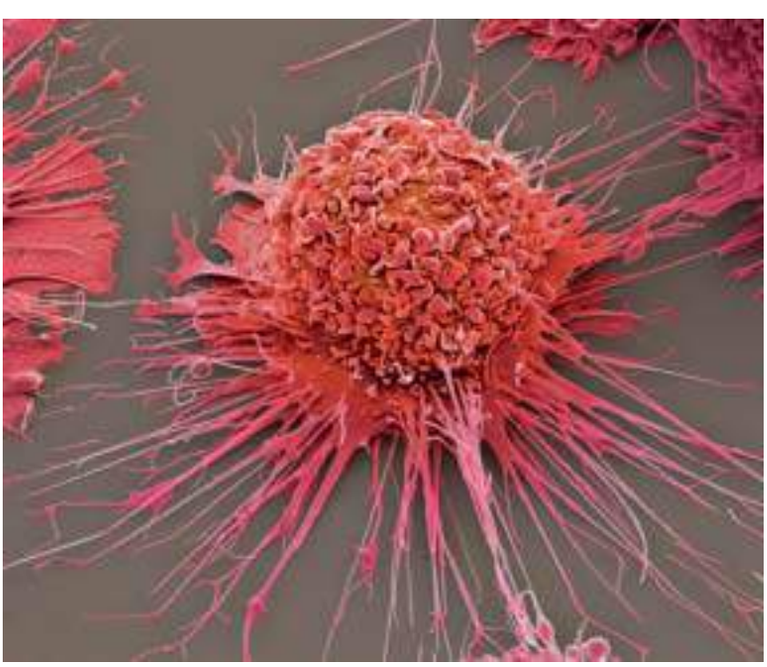
The Importance or the wider use of Scientific imaging

Steve Gschmmeissner
www.theworldcloseup.com

Some of sciences most powerful statements are not made in words.
From the diagrams of Leonardo da Vinci to Rosalind Franklins X rays visualisation of research has a long and illustrious history.
“To illustrate is to enlighten”
SEM images draw the viewer in, showing the microscopic world in a unique and often powerful fashion.
With modern sophisticated imaging technology scientists should aspire to use these images outside the confines of research and medicine.



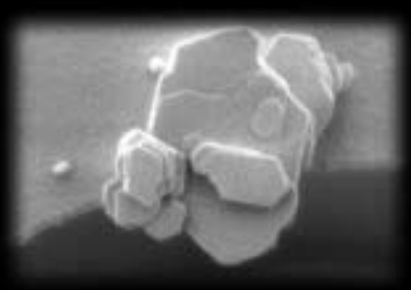
Vitruvian Man
Leonardo da Vinci



Activated macrophage

Hitachi SU8200

with next-generation CFE:
the perfect fusion of
observation & analysis



Kaolin, 50v, 150kx

400nm

Resolve.

Hitachi's revolutionary next-generation CFE gun combines the smallest & brightest source with narrow energy spread and incredible probe current stability – a highly coherent source in every respect.
The result? Incredible resolution from just 10eV.

Detect.

Two in-lens Super ExB detectors with SE/BSE energy filtering show it all, even at extremely low voltages.
Options for BF/DF-STEM and multi-segment BSE complete the most flexible and sensitive detection system available.

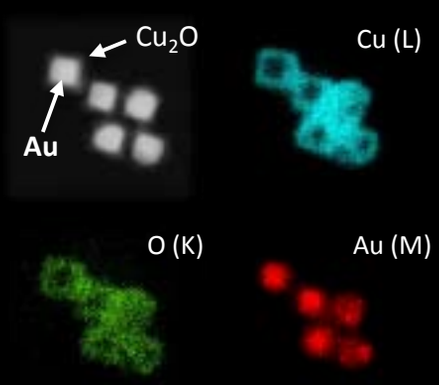
Analyse.

Need to analyse sensitive and nanoscaled materials?
Hitachi's novel CFE gun provides long-term stability and high probe current at all voltages – making high spatial resolution x-ray microanalysis and EBSD routine.

Understand.

Hitachi's SU8200 provides more information, more quickly and more easily than ever before - for deeper sample understanding.

Next-generation CFE – for high performance imaging & analysis.



90nm Cu_2O / Au core-shell nanocubes
5kV, 0.7nA, 15min
Acquired with Oxford Instruments Xmax^{N}150
Courtesy of Prof. Toshiharu Teranishi,
Institute of Chemical Research, Kyoto University

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