The Society of Electron Microscope Technology

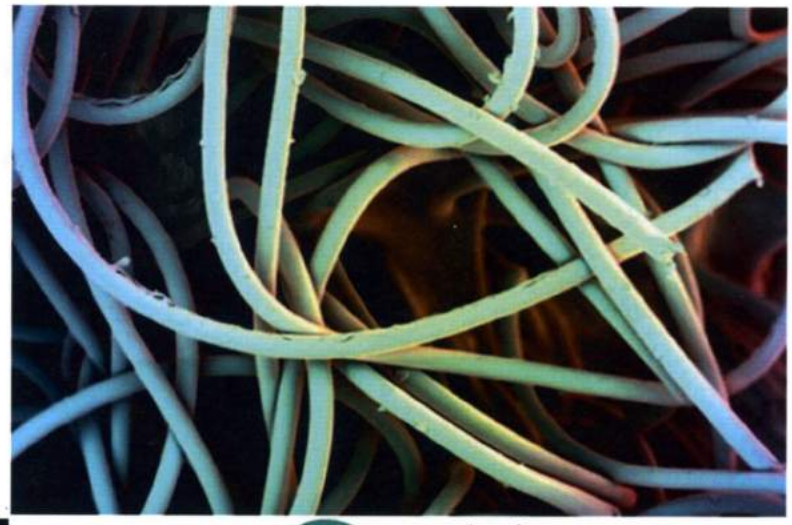
SEMT

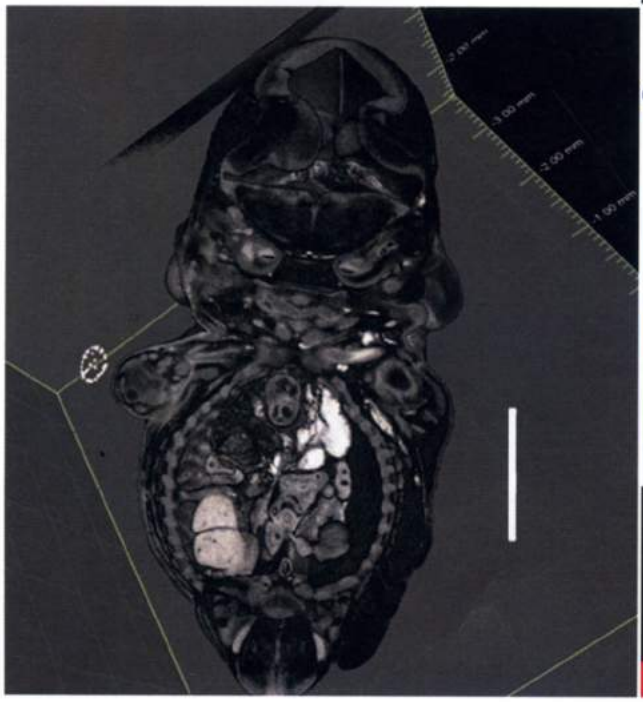
One Day Meeting

Wednesday 16th December 2015

At

The Natural History Museum





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9.00	**Tea/Coffee, Trade Exhibition**
9.40	Introduction: Chair, **Chris Jones**
9:45	**Automated mineralogical SEM as a precursor to trace element analysis by Laser Ablation ICP-MS** **William Brownscombe** - The Natural History Museum
10:20	**Digitisation at the Natural History Museum: Systems for slides** **Rebecca Summerfield** - The Natural History Museum
10:55	Tea, Coffee, Trade Exhibition
11:15	**RMS Beginners Competition**
11:55	**Don Claugher Bursary winner** **Possible cytotoxic effects of pesticide consumption on the midgut of bumblebees (*Bombus terrestris*).** **Cristina Botias** - University of Sussex
12:15	Annual General Meeting
12:30	Buffet Lunch, Trade Exhibition
13:55	**Microscopy and public engagement** **Dr Louise Hughes** - Oxford Brookes
14:30	**Mechanism of daily renewal of photoreceptor outer segments** **Dr Tom Burgoyne** - Institute of Ophthalmology, University College London.
15:05	**3D Imaging of Failure Mechanisms in Electrochemical Energy Devices** **Farid Tariq** - Imperial College London
15:40	Tea/Coffee, Trade Exhibition
16:00	**Have Microscope, Will Travel: Outreach with an SEM is Enrichment for Students** **Dr Thomas Weller** - St. Paul's School
16:35	**ADB Diagnostic and research micro-CT in human anatomy** **Dr Ciaran Hutchinson** - Great Ormond Street Hospital
17:10	Wine Reception
18:30	End of conference

Prospective member can be added to our Members List by contacting.

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Acknowledgments

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Automated mineralogical SEM as a precursor to trace element analysis by Laser Ablation ICP-MS

Will Brownscombe[1]

Eloise M Harman[1], Shaun D Graham[2], Clara C Wilkinson[1], and Jamie J Wilkinson[1]

1- The Natural History Museum, Cromwell Road, London SW75BD

2 - Zeiss Natural Resources Laboratory, Cambridge, CB1 3JS

The LODE (London Centre for Ore Deposits and Exploration) laboratory at the Natural History Museum, London, specialises in trace element mineral analysis with applications for ore deposit research.

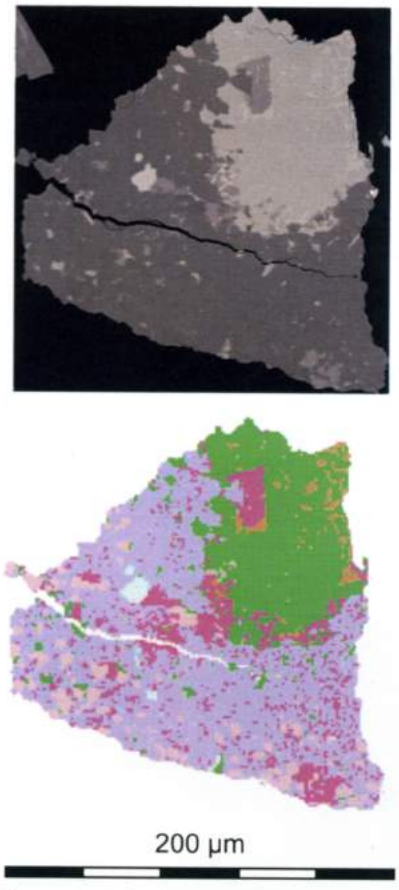

Figure 1 – A backscattered electron image of a mineral grain and an EDS mineral map of the same grain.

This is undertaken using SEM and LA-ICP-MS (Laser ablation inductively coupled plasma mass spectrometry). At present, the SEM is operated manually to characterise the minerals of interest and provide internal standardisation for the LA-ICP-MS technique.

The Natural History Museum has been working with Zeiss to optimise their Mineralogic automation platform for use as a precursor for LA-ICP-MS. Fully- automated mineral identification and quantification has the potential to unlock LA-ICP-MS analysis to allow high throughput analysis and dramatically decrease the staff time used per sample.

The planned workflow consists of backscatter thresholding of a desired range followed by EDS mapping to identify the minerals of interest. The positive identifications are ranked in terms of mineral grain size, reanalysed to obtain fully quantitative data for internal standardisation, and exported along with their XY coordinates for automated running on the LA-ICP-MS system.

Such a workflow is important because it is only with successful automated mineralogy that exploration tools based on mineral chemistry will be economically-sustainable and attain greater penetration in the mining industry.

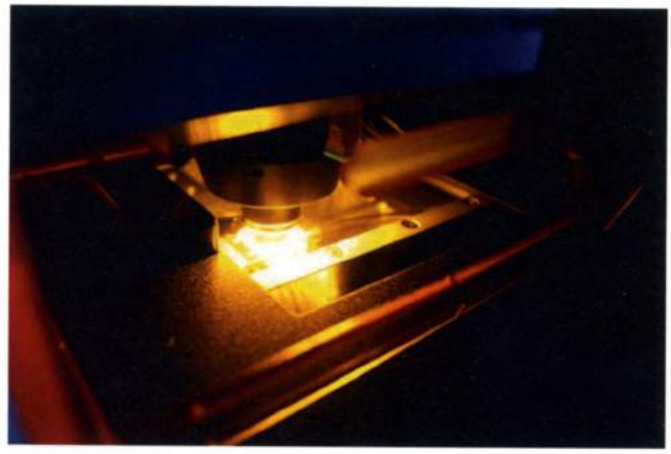

Figure 2 – The LODE LA-ICP-MS ablation cell (NWR 193)

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Digitisation at the Natural History Museum: Systems for slides

Rebecca Summerfield

The Natural History Museum, Cromwell Road, London SW75BD

In museums across the world digitisation initiatives are being pursued in order to audit collections, make data more open and accessible, and bring collections into the modern age. Key to this is the development of new, and the adaptation of existing, infrastructure and systems that can make and store a virtual copy of millions of specimens. At the Natural History Museum (NHM) the Digital Collections Programme is responsible for this and the Slide Digitisation Pilot (SDP) is one of four trials in progress, starting in February 2015 and due to end in April 2016.

The aims of the SDP are to create workflows and data pipelines for high-throughput and high-resolution digitisation of five slide collections. The NHM microscope slide collections are extremely variable. Petrology slides containing microfossils, histological sections, and a range of whole and dissected entomological mounts were chosen in order that systems created are suitable for a as wide a range of slide types as possible.

High throughput workflows have been designed and implemented to collect basic overview images and label data from 100,000 slides using the SatScan Collection Scanner (Smartdrive, UK) (details are given in Figure 1A). Custom written software, Inselect, segments these large overview images into individual slide images and allows basic collection annotation before they are ingested into the collection management system (KE-EMu) via an automated script (Figure 1B). Methods for achieving high -resolution digitisation are still in development. A Zeiss AxioScan (Zeiss, Germany) histology scanner has been adapted to image specimens at 5x magnification and the Zeiss AxioZoom is being used in parallel to this for non standard slides. Final images are then run through an ingest script which uses the barcode number to link these images to the record created during the high throughput workflow in KE-EMu. This talk will discuss the feasibility of using this approach for large scale digitisation.

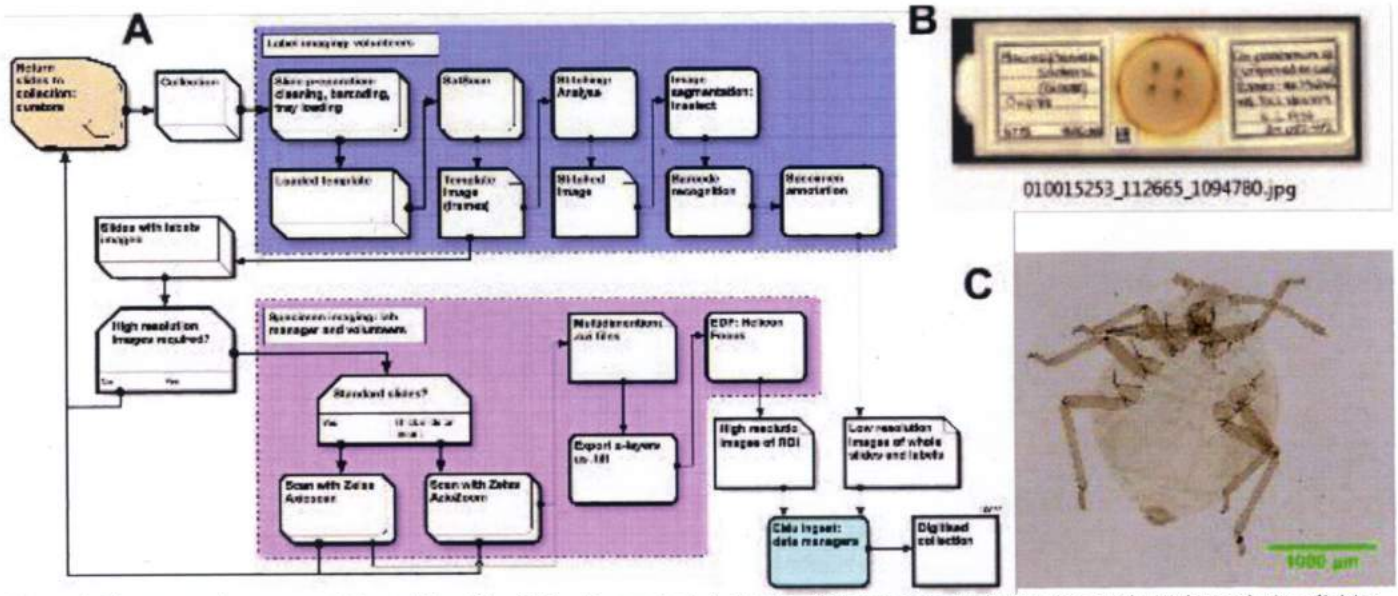

Figure 1. Diagrammatic representation of the slide digitisation project. A: Purple- high throughput digitisation. Pink- High resolution digitisation. B: Final output from high throughput method, note the name of the image it is three strings i- barcode number which is used to create a new record in K-EMu ii- collection location IRN iii- taxonomy IRN these two are utilised by the ingest script to tag the new record to the correct place in the virtual collection, where addtional transcription can be done. C: Final output of the high resolution pathway.

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Don Claugher Bursary award presentation

Possible cytotoxic effects of pesticide consumption on the midgut of bumble-bees (*Bombus terrestris*).

Cristina Botías[1],

Pilar García-Palencia[2], Dave Goulson[1]

[1]School of Life Sciences, University of Sussex, Brighton BN1 9QG, United Kingdom

[2]Facultad de Veterinaria, Dpto. de Medicina y Cirugía Animal, Universidad Complutense de Madrid, Madrid 28040, Spain

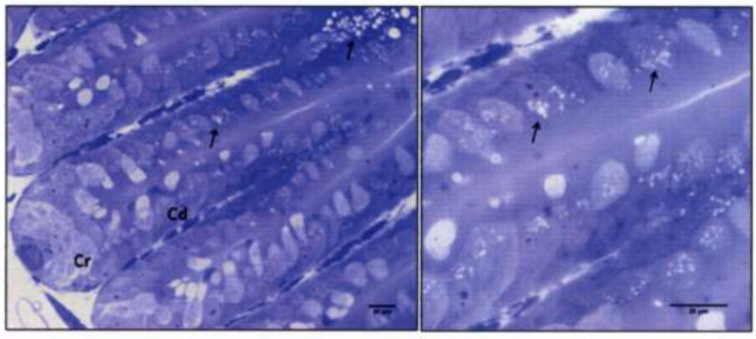
Declines in pollinator abundance have led to fears of a 'pollination crisis' which threatens both agricultural productivity and biodiversity. Bees face many stresses in the modern world, of which pesticides and parasites are widely accepted to be some of the most significant. Moreover, these stressors interact in ways that are not always predictable. In particular, recent evidence suggests that pesticides may impair the immune response of bees, encouraging replication of parasites, and this interaction may be key to understanding health problems being experienced by both wild and managed bees. Neonicotinoid insecticides have shown to cause morphological and histochemical alterations in the digestive and regenerative cells of the midgut from exposed honey bees. Moreover, higher replication of the gut parasite *Nosema ceranae* has been reported in honeybees exposed to this type of insecticides and other agrochemicals. Therefore, it is likely that exposure to these compounds may cause cell damage and an increase in the replication of *N. ceranae* in the gut of wild bee species such as bumblebees as well, but these questions have not yet been addressed. Alterations in the midgut of bumblebees could impair their digestive function and thus, have an impact on their health status. These deleterious effects may have serious consequences on pollination services and the conservation of these important pollinators.

The objectives of this study are (a) evaluating the possible cell damage caused in the gut of bumblebees after consumption of food treated with a combination of agrochemicals that the bees are routinely exposed to in field conditions, and (b) assessing the possible increase in the reproduction rate of *N. ceranae* in the gut of bumblebees exposed to pesticides.

To this end, twenty bumblebee colonies were randomly assigned to 4 different treatment groups (5 colonies per group): Colonies from Group 1 received field-realistic doses of a combination of pesticides, and bees were inoculated with *N. ceranae*, colonies from Group 2 received food with the combination of pesticides, colonies from Group 3 were inoculated with *N. ceranae*, and finally Group 4 was formed by the control colonies.

One bumblebee per colony was collected (N=20) and their guts were dissected and prepared for histopathological analysis using Electron Microscopy techniques.

Preliminary results show that midgut sections under electron microscopy of bumblebees exposed to a combination of pesticides and inoculated with *N. ceranae* (Group 1) presented vacuolization (arrows) in the cytoplasm of numerous cells, but these observations need to be compared with non-exposed bees from the control group. Nosema infection was not detected in any of the sections observed from the bumblebees of Group 1.



(Cr) regenerative cells, (Cd) digestive cells

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Using Micro-CT to investigate whether the cognitive demands of a complex breeding strategy in solitary wasps lead to changes in brain structure

Feargus Cooney[1],

Farah Ahmed[2], Jeremy Field[3] Mike Cant[1]

CLES, University of Exeter

IAC, NHM, London

EBE, University of Sussex

The cognitive demands of complex spatio-temporal learning and memory have been linked to brain development in numerous species, from fruit flies to humans. In insects, studies of these relationships have concentrated mainly on the mushroom bodies, which are specific areas of the brain which tend to be especially enlarged in taxa which display complex life histories. Digger wasps in the clade Ammophilini provide parental care to their developing larvae by provisioning them with food at the time of egg-laying, which they then seal inside an underground burrow during development. Most Ammophilini provide all the food necessary to reach adulthood at the time of laying before abandoning them, while some return repeatedly to reprovision the larvae at various stages during development, and may be maintaining several larvae simultaneously. We are testing whether variation in the complexity of parental care strategies can explain patterns of brain evolution in this system. This study uses Micro-CT scanning to visualise brain structures within an intact specimen. We also address the optimisation of brain tissue staining methods and protocols for measuring specific regions of the mushroom bodies. Future work involves morphometric measurements and principal component analysis to test whether the evolution of complex, extended parental care is associated with larger or more complex brains.

Imaging and visualization of the life stages of Chinese mitten crab using Confocal Microscope and CT Scanning

Seyit Ali Kamanli[1,2,3], Dr David Morritt[1], Dr Paul Clark[2], Dr Alex Ball[3]

[1] School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, UK;

[2] Department of Life Sciences, The Natural History Museum, Cromwell Road, London, UK;

[3] Imaging and Analysis Centre, The Natural History Museum, Cromwell Road, London, UK

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Description of larval characters can provide solutions to many existing problems in brachyuran taxonomy and a detailed description is crucial to make correct identification of planktonic zoeae in marine and freshwater. In this study, Confocal Laser Scanning Microscopy (CLSM) was used to examine zoea stages of *Eriocheir sinensis*. A suitable methodology for CLSM processing including pre-processing and post processing, were applied to visualise these zoea. Confocal microscope data enables three-dimensional reconstruction of the specimens. We use the software programme "Drishti" for this aim and it gives very accurate information on morphology and shows even diminutive structures, such as setae, in detail (Figure 1). Micro CT-scanning is also a very effective tool to visualize similar features of brachyuran crabs (Figure 2). In this presentation I will provide a short description of a developed methodology for the imaging of crustaceans.

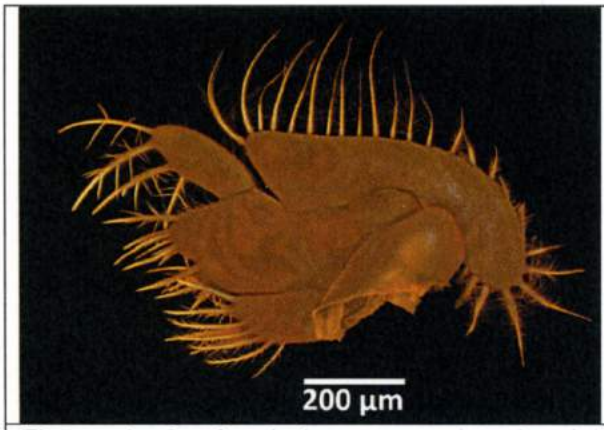

Figure 1. Confocal dataset rendered using Drishti.

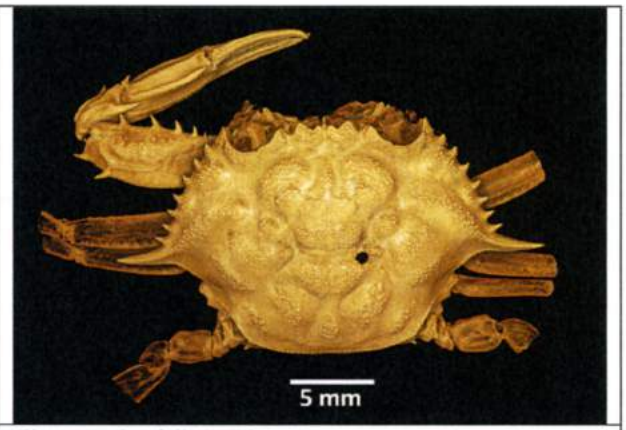

Figure 2. Micro-Ct dataset rendered using Drishti.

Microscopy and public engagement

Louise Hughes

Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, OX3 0BP.

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Public engagement, science communication and outreach is increasing throughout the sciences, both as a way to get younger audiences to engage and potentially enter scientific careers and as a way to inform the public about what research they are funding and why it is important. It is becoming a requirement of some funding agencies to allot a certain amount of time and money to public engagement activities. Microscopy has the ability to engage a wide range of audiences and has fascinated both scientists and the public since the publication of Robert Hooke's Micrographia 350 years ago first bought the microscopic world to everyone's attention. As microscopists, we already have a tool that can be readily understood by the public. At a time when the internet and social media has changed the way in which we communicate, an image really is worth a thousand words!

However, do we have time for science outreach? How can we fit public engagement into schedules that are already stretched? What types of activities can we get involved in and how do we go about running events?

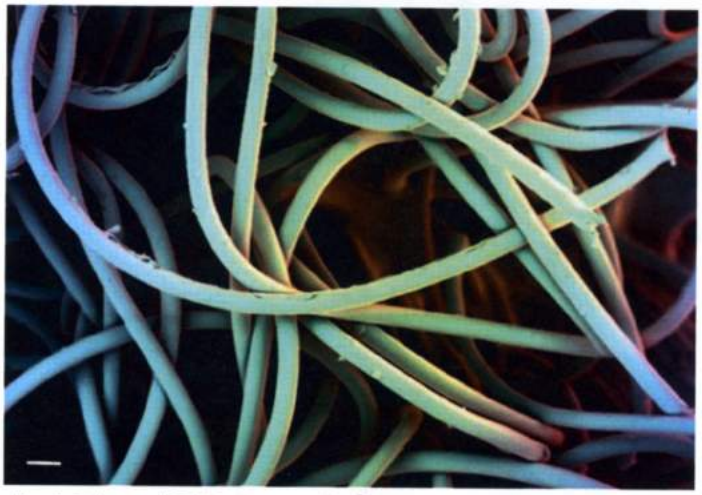

Fig. 1. Coloured SEM image combines BSE and SE signals and shows Velcro mesh. Scale bar = 100µm

Fig. 2. Milly Farrel and Anne Osterrieder with our exhibition stand at the BBSRC Great British Bioscience Festival, 2014

How can we use today's tools to widen participation and reach audiences that we would normally be unable to engage with? I will discuss our successes and the challenges we faced in our explorations into public engagement, what has worked, what hasn't and how to enter into this arena in a variety of different ways.

Fig. 3. Collaborative art exhibition between Oxford Brookes University, the RMS and Rob Kesseler, 2015

Mechanism of daily renewal of photoreceptor outer segments

Thomas Burgoyne,

Institute of Ophthalmology, University College London.

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The specialised light sensing organelle of vertebrate rod photoreceptors cells is a modified cilium known as the outer segment. It contains ~1000 stacked disc membranes that are enriched in the light sensitive pigment rhodopsin. Due to the high metabolic demand of phototransduction, the distal 10 percent of the outer segment is shed every day and replenished at the outer segment base. The disc constituents are synthesised in the inner segment and transported via the connecting cilia to the base of the outer segment to form new discs. The mechanism of transport of disc constituents to the outer segment and how they are assembled into new discs is a subject of intense debate. We have used immuno-electron microscopy and electron tomography of the inner: outer segment interface to generate a 3D model of how rhodopsin transported on the ciliary plasma membrane is assembled into discrete discs at the base of the outer segment.

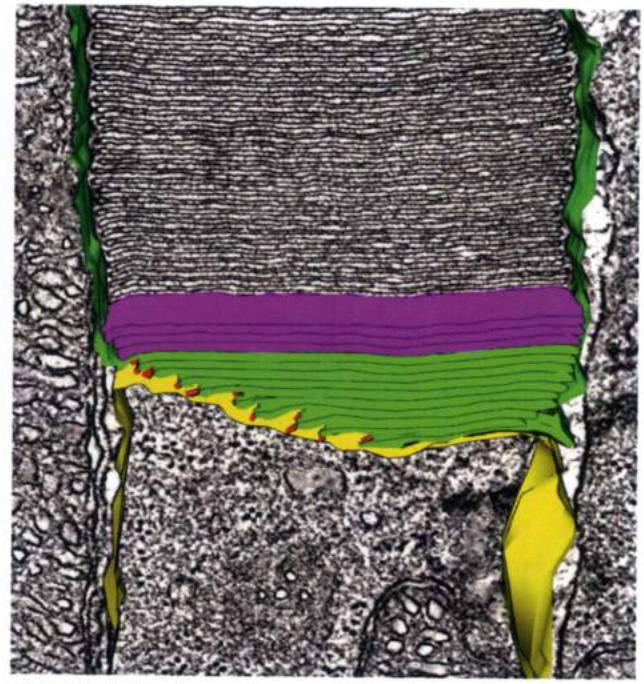

Figure 1. A slice from a tomographic reconstruction of a mouse rod photoreceptor inner and outer segment with a model overlaid.

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3D Imaging of Failure Mechanisms in Electrochemical Energy Devices

Farid Tariq*[1,2],

Vladimir Yufit[1,2], Moshiel Biton[1] , Zhangwei Chen[1] and Nigel Brandon[1,2]

[1]Department of Earth Science and Engineering, Imperial College London, UK

[2]Quantitative Imaging Division, IQM Elements, UK

*Presenting author, email: farid.tariq02@imperial.ac.uk, Tel.: +44 207 594 5124

Meeting increasing energy demands, storage demands and energy portability in a clean efficient manner will be expedited through an ability to directly image energy devices such as fuel cells and batteries. Tomographic techniques allow for the 3D imaging and characterisation of complex electrode microstructures down towards tens of nanometers; which are inadequately described in 2D.

The performance of the electrode is dependent on nano/micro-structure as the electrochemical reactions and transport phenomena are strongly affected by the complex porous microstructure. Furthermore, during processing or operation microstructural evolution may degrade electrochemical performance.

Here we use tomographic techniques to probe the 3D electrode structure at micro-nanometer length scales. Subsequently, micro/nano structural changes are followed to facilitate understanding the differences which occur with shape, structures and morphology at high resolution. We develop both ex-situ and in-operando 3D imaging techniques, modelling and 3D quantitative analysis of electronic, ionic and gas interfaces (Fig 1); which are correlated with observed differences in electrode behaviour. Through an understanding of the sources of failure mechanisms, we can optimise and begin to manufacture electrodes by design.

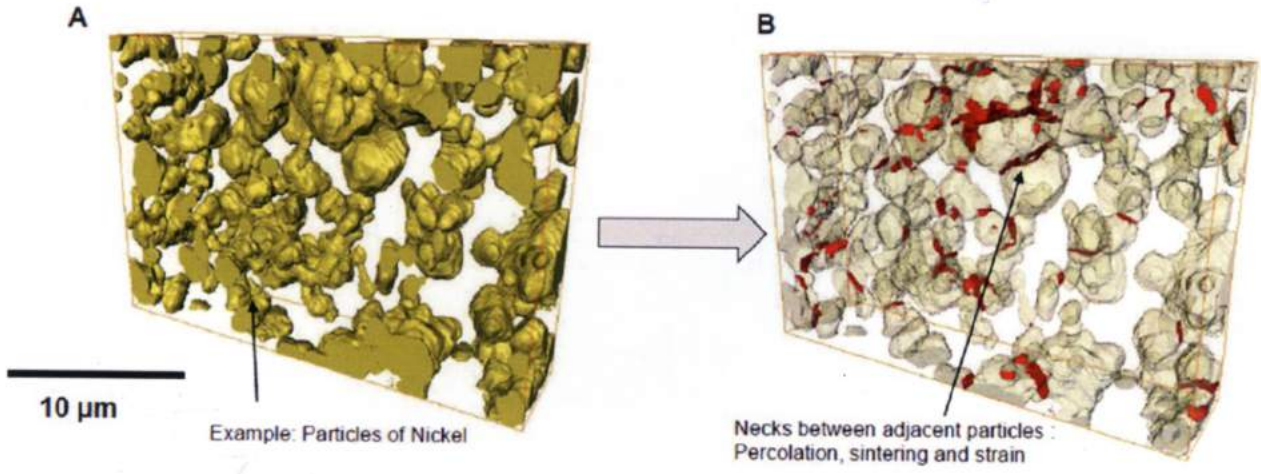


Fig. 1 – Identification and quantitative analysis of electronic, ionic and gas interfaces

Have Microscope, Will Travel: Outreach with an SEM is Enrichment for Students

Thomas Weller

St Paul's School, Lonsdale Rd, London SW13 9JT

mailto:TEW@stpaulsschool.org.uk

A portable SEM is an accessible research tool, both for a scientist and an interested member of the public. It provides images with extraordinary depth of field, as well as technical information on morphology and composition. Our Hitachi TM3030 portable SEM is so designed as to be robust to novice users with minimal supervision. I am happy to stand and watch a child navigate and zoom around a sample, as I have done at both the Lyme Regis Fossil Festival and Science Uncovered.

Lyme Regis and Science Uncovered fit well into our Outreach and Enrichment programmes. Outreach in sharing our resources, enrichment in giving our students an opportunity in science communication. The SEM is a crucial part of our aim to conduct scientific research in school; and of course, science communication is a crucial part of research.

This talk will describe our experiences at Lyme Regis Fossil Festival and Science Uncovered. The Fossil Festival is held around the beginning of May in a giant single story tent on a wooden platform on the beach, very noisy conditions with 1000s of visitors each day tramping the boards, and many hands on activities. The Science Uncovered event is held on firmer ground in the Natural History Museum, in late September, with similarly hands on activities and a much wider theme. We will describe the process of set-up, whilst we set up live, and then show you two of our approaches. The approaches are both structured, but allow for improvisation and development. We developed them at the events. No doubt there is more to learn. We hope that this talk will stimulate discussion about the blending of outreach and enrichment for students and staff, not least in communication training for budding scientists.

Medical applications of Micro-Computed Tomography: New Diagnostic Possibilities

J. Ciaran Hutchinson,

Owen J Arthurs, Andrew T Ramsey, Michael T Ashworth, Neil J Sebire

Great Ormond Street Hospital

Ciaran.Hutchinson@gosh.nhs.uk

Microscopic dissection of small histological specimens is technically difficult, prone to error in sampling and interpretation, and by its very nature, destroys the tissue under examination. The ability to acquire images with resolutions similar to a light microscope in autopsy cases and surgical biopsies would represent a significant improvement in practice. We present a series of cases examined using micro-CT including forms of congenital heart, lung and kidney disease, with a direct comparison of histological appearances.

Specimens were immersed in potassium tri-iodide prior to micro-CT examination. Images were acquired using micro-CT scanners with a multi-metal target system (Nikon Metrology, Tring, UK). Scans were reconstructed using proprietary Nikon software (CTPro3D) and processed using VG Studio MAX (Volume Graphics GmbH, Heidelberg).

All specimens demonstrated excellent internal tissue contrast and all micro-CT scans acquired provided the necessary level of detail to make an accurate morphological diagnosis without the need for further dissection. In the case of congenital heart disease, some features were better identified on micro-CT examination than at macroscopic dissection.

These data show that micro-CT has the potential to provide clinically useful imaging information, with visualisation similar to that of conventional histopathology. Micro-CT has the additional advantage of creating a permanent 3D dataset that could be sent for second opinions as well as allowing pre-dissection analysis of the correct dissection approach and potential assessment of surgical margins. Traditionally, pathologists infer diagnostic information about three-dimensional pathological processes occurring within an organ from 2-dimensional glass slides; with further optimisation, micro-CT may become a routine step in the analysis of complex specimens where volume relationships may alter clinical diagnoses. The addition of micro-CT imaging to our workload of 5000 histopathological examinations and 500 autopsies performed at our institution per year could represent a significant improvement to clinical services. Micro-CT has the potential to change the way in which early fetal miscarriages and terminations (<24 weeks gestation) are performed and recorded.

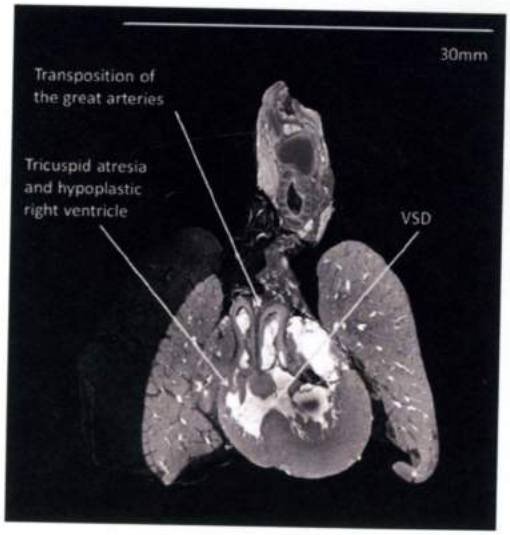


Figure 1 – Hypoplastic right heart syndrome, 18 gestational weeks.

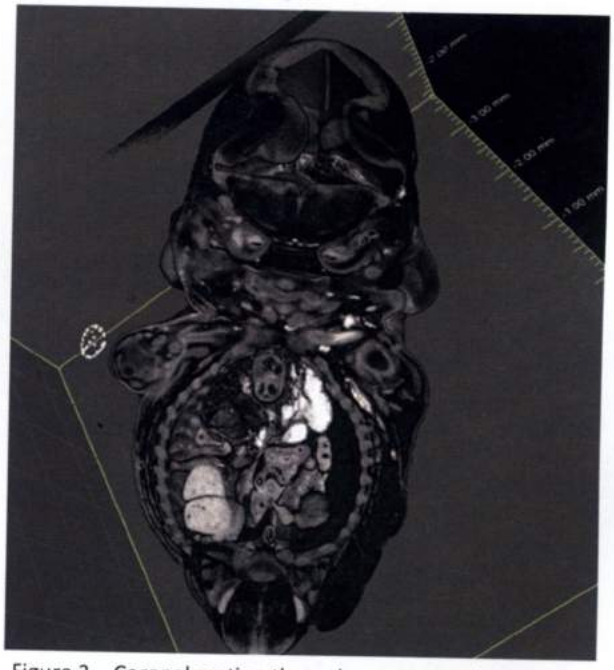
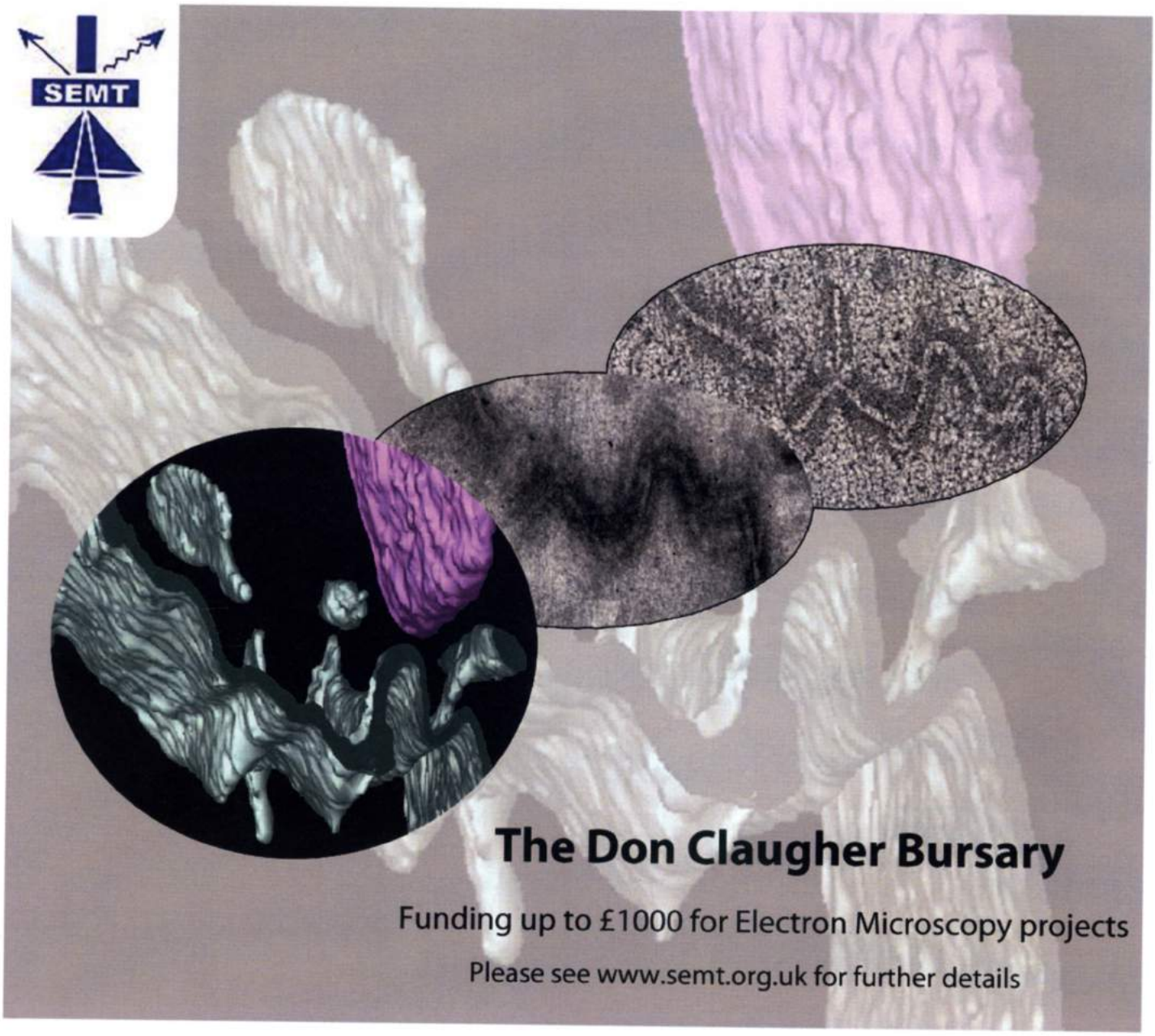


Figure 2 – Coronal section through a mouse embryo (E13.5), pulmonary and aortic valves are seen. White bar = 1mm.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The Society of Electron Microscope Technology

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