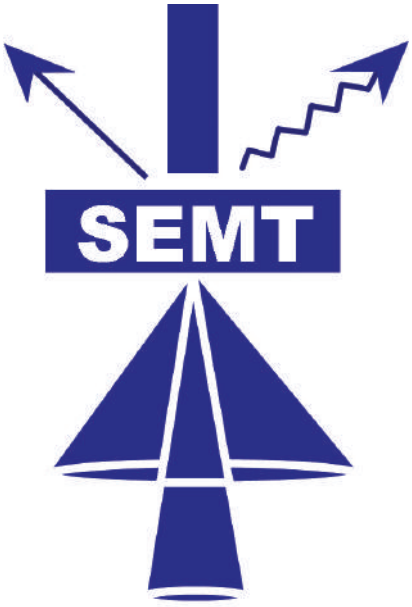
Keeping you informed of the latest technology & techniques

Society of Electron Microscope Technology



SEMT
One Day Meeting

Wednesday 10th December 2008

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
Mrs. 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, 9th December 2009.

9.15 am	Registration, Tea/Coffee, Trade Exhibition
9.55 a.m	Introduction: Chair, Heather Davies.
10.00 am	**The diagnosis of a vascular neurodegenerative disease.** **Ray Moss.** ST Georges Medical School London.
10.35 am	**Molecular genetic work is not only related to the electron microscopy but also dependent on it.** **Lu Liu.** The National Diagnostic EB Lab, StThomas' Hosp. London.
11.10 am	Tea, Coffee, Trade Exhibition.
11.30 am	**"Stem cells in Huntington's disease"** **John Golding**. Dept. of Health Sciences, Open University.
12.05pm	**"The Application of Microscopy in the Aerospace Composites Industry"** **Trevor Groves.** Hexcel, Duxford, Cambridge.
12.40pm	Buffet Lunch, Trade Exhibition.
2.30 pm	**"Understanding Image Formats in the context of Electron Microscopy".** **Andy Yarwood.** Jeol UK Ltd, Welwyn Garden City, UK
3.05 pm	**'Measuring mechanical properties at the nanoscale using electron and scanning probe microscopy'** **Asa Barber.** Dept. Materials, Queen Mary, London.
3.40.pm	Tea, Coffee.
4.00 pm	**The Use of Thermal Imaging in Forensic Entomology Research** **Amoret Whitaker.** Department of Entomology, Natural History Museum, London.
4.35 pm	**CT Imaging of the inner ears of Reptiles and Birds** **Stig Walsh.** Department of Mineralogy, Natural History Museum, London
5.10pm	AGM - Wine Reception
6.30pm	Conference Dinner

Trade Exhibitors

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, RMS, Tron-Tech Ltd, Taab Laboratories Ltd & Zeiss SMT.

The diagnosis of a vascular neurodegenerative disease.

Ray Moss
ST Georges Medical School London

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) leads to stroke and dementia in humans. Diagnosis can be made using gene screening, Immunocytochemistry or electron microscopy as the sensitive diagnostic tool. Skin biopsies were compared to show the varied results and provide a reliable diagnostic marker.

Molecular genetic work is not only related to the electron microscopy but also dependent on it.

Lu Liu
The National Diagnostic EB lab, St. Thomas' Hosp. London

Ultrastructure study with transmission electron microscopy in medical diagnostic field have generated vast amount of fascinating and important results. Molecular genetic knowledge and technique has been used to understanding the mechanisms of genetic inherited diseases. In The National Epidermolysis bullosa Laboratory, we have confirmed that non of these two method alone can answer all the questions that needed to be addressed in order to establish correct diagnosis for EB patient. However, the combination of these two very powerful methods can provide a comprehensive solution to help patient.

“Stem cells in Huntington’s disease”

John Golding
Dept. of Health Sciences, Open University

“Huntington’s disease is a fatal neurodegenerative condition, characterised by the pathological progressive addition of glutamine repeats to the Huntington protein (huntingtin). In the brain, this leads to the accumulation of huntingtin-rich inclusions within neuronal nuclei and neuronal death.

Stem cell therapies are being considered as one way of repopulating the injured brain in several neurodegenerative diseases. However, the potential of using autologous stem cell transplants has not yet been investigated for Huntington’s disease. My work uses a mouse model of Huntington’s disease to examine the properties and potential therapeutic utility of stem cells harvested in the context of the disease.”

“The Application of Microscopy in the Aerospace Composites Industry”

Trevor Groves
Hexcel, Duxford, Cambridge

Aerospace composite and adhesive materials have been manufactured on the Duxford site for 75 years and Hexcel Duxford has the proud pedigree of being involved in the manufacturing programs of many iconic British aircraft including Spitfire, Mosquito, Harrier, Concorde, Nimrod, Vulcan, Tornado and latterly Typhoon Euro Fighter as well as the Airbus A380, the worlds largest passenger aircraft.

Underpinning the successful innovative development and application of these strong light weight materials is the use of microscopy in its many forms. Manufacturing, Quality Control and Research Departmentsthroughout Europe and the US depend to some degree on the application ofmicroscopy whether it be the use of techniques within Hexcel or usingtechniques which are readily available at many institutions external toHexcel.

Understanding the failure mechanisms, the distribution of polymers andthe microstructure of carbon fibre parts, is essential to understandingthe best way to drive continual improvements into products which areused in ever more demanding applications and environments not only in aerospace but other large market sectors such as wind energy,automotive, motor sport, marine, satellite and sports and leisure.

Understanding Image Formats in the context of Electron Microscopy

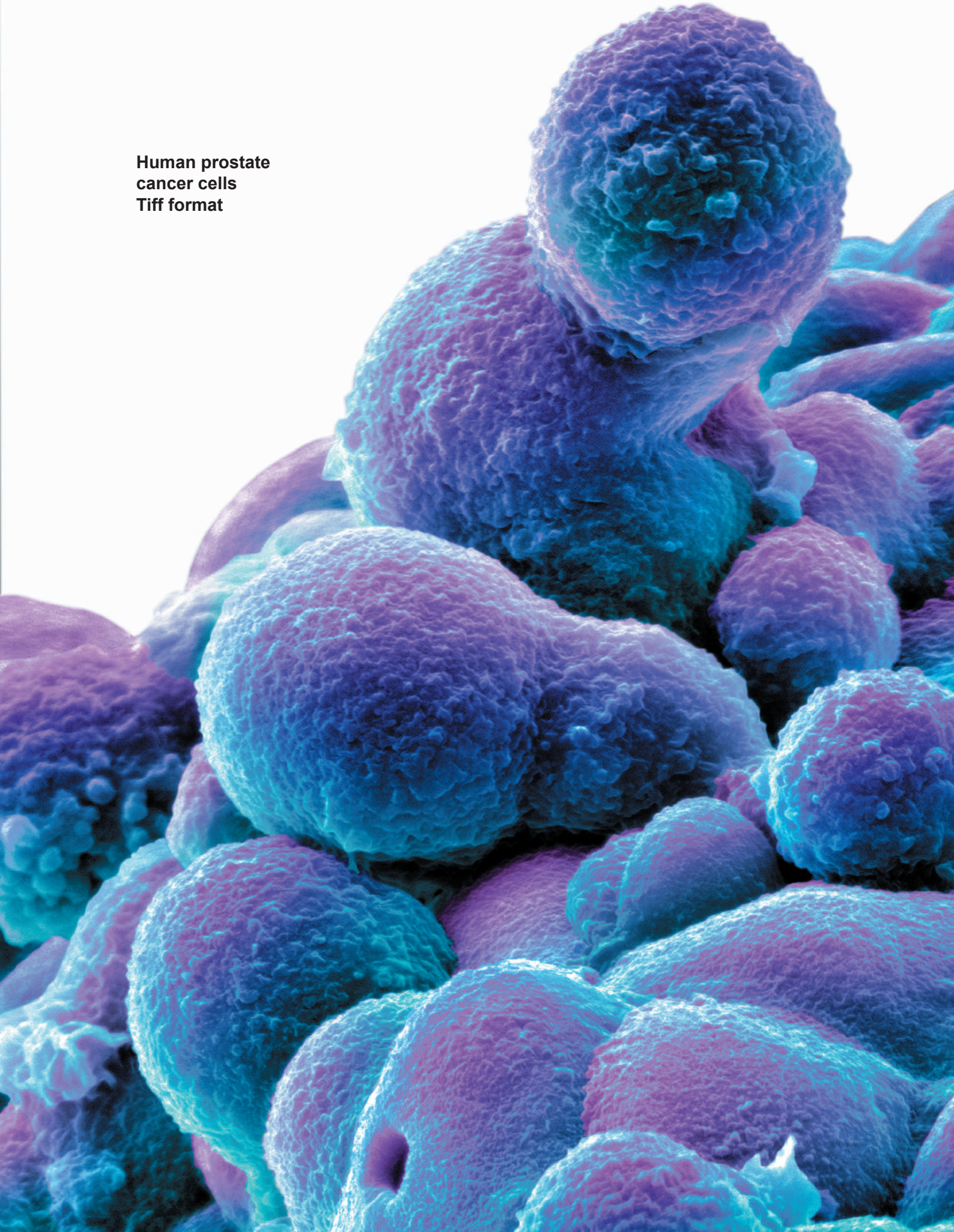
Andrew Yarwood
JEOL (UK) Ltd, Welwyn garden City
Applications Specialist TEM & SEM

There are 3 main image formats commonly used in the Electron Microscopy industry. While 2 of these formats are remarkably similar, namely the bitmap (BMP) and tagged image file (TIFF) formats, the Joint Photographic Experts Group (JPEG) format is totally different in its structure and the way in which it handles what may be valuable image data.

The simplest bitmap formats, including TIFF, store a single image in a variety of in colour or monochrome rendering depths and will consist of a header describing the image dimensions and precision, followed by the image data itself. Unless the data is in some way compressed, the data will consist of a continuous string of data values for each pixel which will be positioned sequentially in the image array defined by the header.

The Windows™ bitmap format has limited options, but is effective in many electron microscopy applications. However the bitmap format has some limitations when it comes to many TEM cameras and modern SEMs. Typically these will produce greyscale data which requires saving into 16-bit files. 16-bit files are not supported by this format. Unfortunately the 24-bit bitmap format does not help as it simply uses 3 8-bit greyscale images stored in an RGB data array. Although the resulting greyscale compromise may be a problem in our minds, the reality is that the human eye cannot see this many grey levels anyway. The advantage of the Windows™ bitmap format is in its efficiency and speed, a fact which many users overlook.
The tagged image file format is generally more favoured by electron microscopists due to its greater range of options and compatibility with many different computer platforms. Many scientific formats are derivatives of the TIFF format, with modified headers to store private data tags and extra image layers if necessary. Ironically TIFF can also be a problem for users of data from TEM cameras in particular due to the fact that 16-bit greyscales are not natively supported by many PC's. Unwary recipients of TEM 16-bit images may be surprised by the apparent lack of image when the file is initially opened, assuming they have a compatible 16-bit greyscale application.
JPEG files are very popular, due to their ability to compress the image data fairly efficiently. The JPEG method should be avoided as the primary data storage format for electron microscopy owing to the fact that the compression algorithm will lose data. Electron microscopy may be a visual technique, but as measurement is becoming increasingly more important the JPEG format may contribute to errors if not controlled properly. Another factor is that the JPEG compression is repetitive, and can destroy an image if it is repeatedly saved from an application during routine image processing. It is interesting to study this effect of the JPEG algorithm on a simple pattern as it helps users to understand how their images will be changed by the JPEG format.
Image formats can be studied to almost any depth, but with the correct information relevant to the science, in this case electron microscopy, users should be able to decide for themselves which image formats are most appropriate.

Human prostate cancer cells
Tiff format



Measuring mechanical properties at the nanoscale using electron and scanning probe microscopy

Asa H. Barber
Department of Materials, School of Engineering & Materials Science
Queen Mary, University of London

Scanning probe microscopy (SPM) was first developed primarily for imaging of surface topography to atomic resolution. More recent developments have used SPM to apply forces to points on a sample and use imaging capabilities to ascertain the deformation post-testing. The application of forces using SPM is conceptually simple; a SPM tip attached to a freely bending beam, know as the cantilever, is pushed towards the sample using piezo-electric manipulators. Forces acting on the tip cause a corresponding bending of the cantilever. Calibration of the cantilever to give its spring constant therefore allows conversion of bending into force. SPM is being currently employed to mechanically deform a number of samples, especially in nanotechnology and life sciences where mechanical behaviour of small samples such as proteins or nanomaterials are unknown or differ from macroscopic measurements. However, SPM is limited in monitoring capacity as the SPM tip must provide both imaging and application of force. This results in experiments typically involving imaging of the sample, deformation of the sample and subsequent imaging to ascertain the result of the test. The connection between the force applied and the deformation mechanism is therefore subjective as no direct information during the deformation processes is possible.

Potential solutions have been suggested by using the imaging capabilities of scanning electron microscopy (SEM) in conjunction with SPM as applying mechanical testing. Initial experiments used a combination SEM and SPM to deform samples in the chamber of the electron microscopy. Thus, force would be recorded from cantilever bending while the SEM provided imaging. The drawback in this procedure is highlighted in the way cantilever bending is recorded in this technique compared to conventional SPM. Reflected laser light from the back of the cantilever to a photodiode in SPM gives sensitive measurements of bending of the order of 1 nm. However, combination SPM within the SEM relies of cantilever bending being recorded by the SEM itself. This results in inaccuracies due to imaging resolution and a laborious method of collecting a series of SEM images to manually deduce cantilever bending. A novel combination SPM-SEM method is presented here which provides continuous recording of cantilever bending from the measurements using an optical interferometer setup.

Combination SPM-SEM

A custom built SPM is integrated within the chamber of a commercial SEM. The interferometer device provides accurate measurement of a calibrated cantilever during mechanical deformation. A polymer nanofibre with a diameter of approximately 100nm is selected to demonstrate the capability of the combination SPM-SEM system. The nanofibre is first manipulated within the SEM chamber so that attachment is achieved between a small amount of glue on the SPM tip and a larger droplet of glue as shown in Figure 1. After waiting for the glue to solidify, translation of the SPM tip away from droplet causes bending of the cantilever as the nanofibre is placed under tension. This proceeds until the nanofibre fails. Continuous monitoring of the cantilever deflection gives the stress in the nanofibre while controlled tip displacement provides a calculated strain. Thus, a stress-strain curve, the staple of mechanical testing in materials science, is produced as shown in Figure 2. Our results show that the elastic modulus of the nanofibre, given by the gradient of the data, shows two distinct regions. The first suggests a soft region followed by increased resistance to deformation at larger strains.

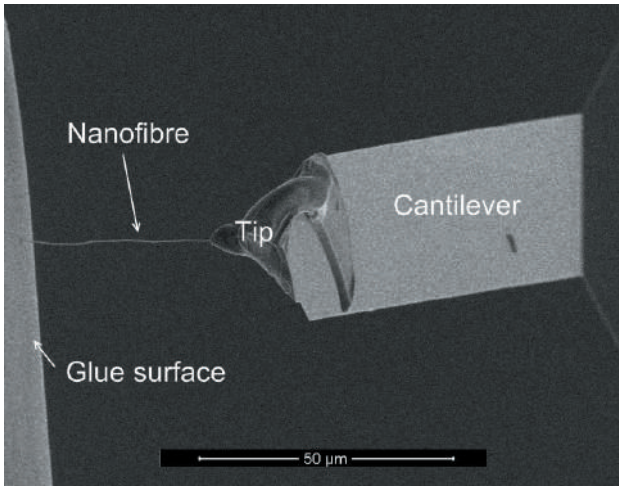


Figure 1. SEM image of the SPM setup used to tensile test an individual polymer nanofibre

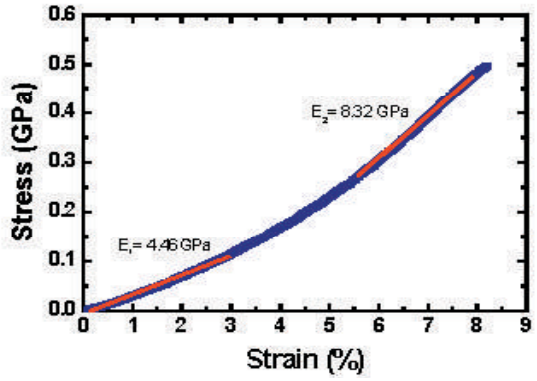


Figure 2. Resultant stress-strain curve produced from tensile testing of an individual polymer nanofibre.

This response has been observed in many macroscopic biological materials such as tendon where the mechanism is explained as initial removal of crimping and increased molecular alignment to cause an increase in the elastic modulus. The combination SPM-SEM therefore gives an unprecedented level of force resolution capable of controlled deformation of small samples and in-situ monitoring for elucidation of the extent of deformation and the resultant failure mechanism.

The Use of Thermal Imaging in Forensic Entomology Research

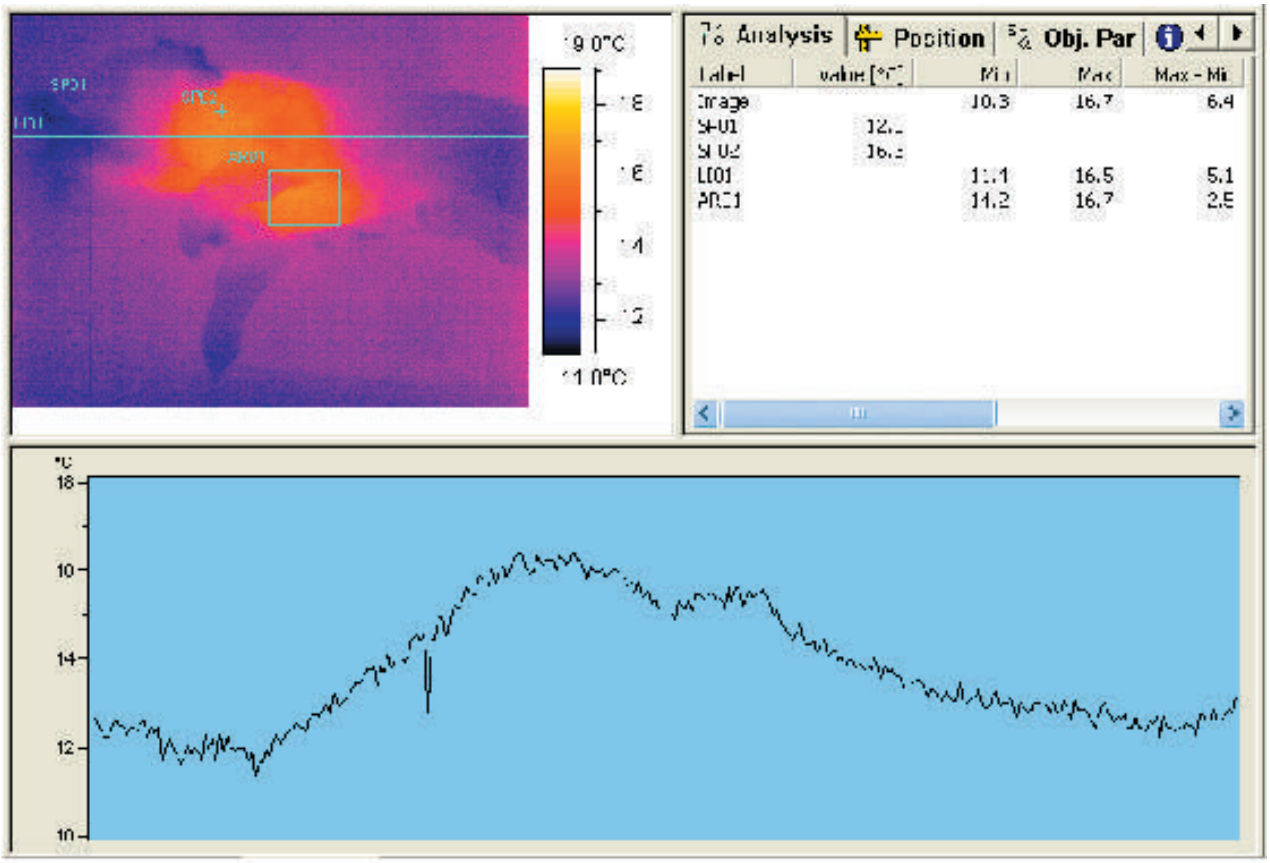
Amoret P. Whitaker
Department of Entomology
Natural History Museum, London.

Forensic Entomology is the study of insects and other arthropods in a legal context. There are numerous areas in which it can be applied, the most high profile being the use of cadaverous insects to estimate the time since death in cases of unexplained or untimely death. Blowflies (Diptera: Calliphoridae) are the most important insects from a forensic perspective because they are the first insects to be attracted to, and to colonise, a fresh cadaver, and therefore can give the most accurate information regarding time of death. Their rate of development is dependent on the temperature under which they develop, thus warmer temperatures will speed up their development and colder temperatures will slow it down. Therefore if the local temperature and the stage of development of the insects can be determined, an estimation can be given for the minimum time since death.

However, there are many factors which will influence the insects' development, the most uncertain of which is the elevated temperatures caused by "maggot masses". When fly larvae reach the late 2nd, and throughout the 3rd instar stage, they commonly feed in aggregated groups, called larval (or maggot) masses. The core temperature of these masses is usually elevated above the ambient temperature, and studies undertaken in Knoxville, USA on human cadavers, have recorded temperatures of 42°C within the larval masses, an elevation of 20°C above the ambient temperature. Therefore for some period of time during their development, the larvae are being exposed to temperatures higher than that of ambient, thus affecting any estimation of post-mortem interval. Although the existence and potential effect of elevated larval mass temperature are well-known, it has been poorly studied and has not yet been quantified to an accuracy which would allow it to be accounted for in post-mortem interval estimates.

Recent studies by the Forensic Entomology research group at the Natural History Museum have begun using thermal imaging, a technique novel to this area of science, in order to study the dynamics of larval masses, the elevated temperatures that result from them, and the thermal benefits of feeding in a larval mass. Studies have involved the filming of: a) individual fly larvae at different densities; b) small piglets manually infested with fly larvae in the laboratory; c) human cadavers naturally colonised with fly larvae in a natural outdoor environment in Tennessee, USA.

The method of using thermal imaging has a number of benefits over traditional methods of recording temperatures: a) it is non-invasive, and therefore does not disturb the larval mass or the cadaver; b) temperature can be recorded over a large area, rather than just being focussed on one small spot; c) temperatures can be recorded continuously over a period of time, rather than just taking a single measurement; d) the temperature of individual larvae can be recorded and their movement can be tracked; e) the thermal data can be recorded, analysed and presented robustly. In addition, thermal imaging could be used at crime scenes to locate concealed bodies and hidden larval masses and hotspots, without contaminating or disturbing the crime scene or the body. The only disadvantages are: a) the cost of renting or purchasing the equipment and software, and b) if the thermal differences are negligible, it is difficult to differentiate between the larval masses and the substrate.



Thermal image of a piglet, showing the elevated temperature of the larval mass present in the neck/shoulder region. The table on the right shows the temperatures of the two spots (SP01 and SP02), the boxed area (AR01), and the line along the body (LI01), the latter also illustrated in the bottom graph.

Estimating hearing and vocalisation in birds and reptiles from the bony anatomy of the inner ear.

Stig Walsh
Department of Mineralogy, EMMA Section, The Natural History Museum, London.

Since it was installed in February this year the new micro-CT facility at the Natural History Museum has been put through its paces with a remarkably wide variety of materials. Here, I demonstrate how micro-CT approaches can be used to answer biological questions by reporting the results of the first project to have been successfully completed by staff at the lab. Braincase structures that housed the auditory and vestibular apparatus are frequently preserved in fossil material, but are rarely described. Some authors have attempted to infer hearing and vocalisation capabilities of extinct taxa (e.g., hadrosaurid dinosaurs) by comparing cochlear duct dimensions of fossils with those of living species. However, the internal space of the endosseous cochlear duct in living reptiles and birds is occupied by soft tissues (e.g., perilymph) other than the hearing organ (the basilar papilla, analogous to the mammalian Organ of Corti). The dimensions of the endosseal cochlear duct therefore may not reflect accurately the dimensions of the basilar papilla itself, bringing into question some inferences about hearing and vocalisation drawn from the structure. However, the relationship between the bony anatomy of the inner ear and hearing (e.g., range of best hearing) has never been rigorously tested in either extant or fossil taxa.

To investigate this, simple endosseous cochlear duct measurements derived from micro-CT analysis were used to construct models of vocalisation, sociality and environmental preference in living reptiles and birds. Fifty-nine extant taxa representing turtles, crocodiles, lizards, snakes, eight avian orders and the tuatara, were selected on the basis of whether they vocalise and on their vocalisation complexity. After scanning, virtual endocasts of the endosseous cochlear duct were digitally segmented, and measurements of length, rostrocaudal and mediolateral width, and volume were taken and scaled to basicranial length. These data were subjected to multiple regression analysis along with hearing mean sensitivity and range, and broad measures of vocal complexity, sociality and habitat preference. Hearing range and mean sensitivity were found to strongly positively correlate with endosseous cochlear duct length. Endosseous cochlear duct length also positively correlated with vocal and social complexity, but negatively correlated with aquatic habitats. These results suggest that endosseous cochlear duct length can be used to predict mean hearing frequency/range in fossil taxa, and that this measure may also predict vocal complexity and large group socialisation given sufficient data to form a comprehensive model.