SEMT

Society of Electron Microscope Technology

ONE DAY MEETING

Wednesday 28th March 2001

School of Pharmacy
Brunswick Square
London WC1N 1AX

The Society of Electron Microscope Technology

Prospective members should obtain an application form from the Hon. Secretary
Mr. David McCarthy, School of Pharmacy, Brunswick Sq. London WC1N 1AX
44 (0) 20 7753 5806; Email: David.McCarthy@cua.ulsop.ac.uk (The annual subscription is £10.00.)
Our web site : www.semt.org.uk
Current committee members are listed below and are available for further information.

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Acknowledgments:
The SEMT wishes to express special thanks to :
The School of Pharmacy as host.
Hitachi Scientific Instruments for contributing Wine prior to conference dinner.

And to the following companies for supporting the Trade Exhibition :
Agar Scientific Ltd
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Future Programme:

Wednesday 16th May 2.00pm	1/2 Day Meeting/workshop	The School of Pharmacy
Wednesday 12th Dec. 2.00pm	1/2 day meeting : Diagnostic EM	The School of Pharmacy
	A Visit is planned for October	

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Programme

9.15- 9.55.	**Registration,** Tea/Coffee. Trade Exhibition.
9.55-10.00	**Introduction:** Chair, *Heather Davies.*
10.00-10.30	**Distribution of GFAP in human brain using Confocal Fluorescence and Immuno-gold localisation under the TEM.** *David Robertson. Institute of Cancer Research, Sutton, Surrey.*
10.30-11.00	**In VitroConfocal and 2-Photon microscopy, the bridge to Electron Microscopy** *Jeremy Skepper. Multi-Imaging Centre, University of Cambridge.*
11.00-11.30	**Coffee.**
11.30-12.00	**Targeting GFP and other fluorescent proteins to the Golgi and using Photobleaching to look at transport in the secretory pathway.** *Chris Hawes. School of Biological & Molecular Sciences, Oxford Brooks University.*
12.00-12.30	**Digital Imaging and Image Management in Microscopy.** *Paul Wetton. Imaging Associates, Thame, Oxford.*
12.30-2.30	**LUNCH. Trade Exhibition and judging of Posters.**
2.30-3.00	**Low Vacuum vs Low Voltage applications in the ESEM FEG.** *Steve Habesch. Materials Research Institute, Sheffield Hallam University.*
3.00-3.30	**Applications of Cryo-TEM in Modern Molecular Biological Research.** *Brent Gowen. Centre of Structural Biology, University of Oxford.*
3.30-4.00	**Tea.**
4.00-4.30	**A Novel Stereological Approach to Quantifying Intracellular Immuno-gold Labelling Patterns.** *Terry Mayhew. School of Biomedical Sciences, Queens Medical Centre, University of Nottingham.*
4.30-6.15	**Close, followed by Wine Reception.**
6.30.	**Conference Dinner,** *Local Restaurant.*

Abstracts

Distribution of GFAP in human brain using Confocal Fluorescence and immuno-gold localised under the TEM

David Robertson. Institute of Cancer Research, Sutton, Surrey

In this presentation a-GFAP (anti Glial Fibrillar Acidic Protein) is used to label Glial cells in fixed brain tissue, using 25um sections cut on a vibrating microtome, for confocal microscopy. Small pieces of the same tissue (2mm) fixed in the same way but processed through into Lowicryl HM20 resin by the PLT technique (progressive lowering of temperature) and polymerised at -50°C are used to localise GFAP by immuno-electron microscopy.

In vitro Confocal and 2-photon excitation Microscopy: the bridge to electron microscopy.

J.N.Skepper, Multi-Imaging Centre, University of Cambridge.

The difference in resolution from the light microscope (400 x 400 x 600nm) to the electron microscope (< 0.1nm) used to be described as the "yawning gulf". *In vitro* physiological imaging techniques using the confocal and TPE microscopes do much to bridge this gap. Neither technique can improve upon the theoretical resolution of the light microscope but both provide information unobtainable by electron microscopy alone. Paradoxically it is possible to see structures (e.g. microtubules) that are smaller than the resolution limit, simply because they fluoresce so brightly that their signal is higher than background noise. However, measurements of the dimensions of such organelles will inevitably be overestimations. Complex de-convolution algorithms can correct these discrepancies to a certain extent but they are pronounced in unprocessed mages. A simple example of this phenomenon is the estimation of the volume fraction, of junctional sarcoplasmic reticulum in insect flight muscle. Estimates from moderately thick confocal, optical sections (0.75 um) produce 2 or 3 fold higher values than estimates from thin sections (50 nm). Confocal and TPE microscopes excel in imaging dynamic processes in living cells or tissues. However, the electron microscope still has a highly significant role in extending the interpretation of such data. We have used confocal and TPE microscopy to visualise oxidative stress in endothelial cells and evoked calcium waves and sparks in skeletal muscle. Subsequent ultrastructural and immunogold labelling studies have been essential to a fuller interpretation of these studies. The two disciplines are both essential but different facets of microscopy and complement one another rather than compete.

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Targeting GFP and other fluorescent proteins to the Golgi and using photobleaching to look at transport in the secretory pathway

Chris Hawes, Hugo Zheng, Claude Saint-Jore, Alexandra Andreeva and Fede Brandizzi Research School of Biological Sciences

In higher plants the relationship between the endoplasmic reticulum (ER) and Golgi apparatus (GA) is not well characterised. The advent of the green fluorescent protein (GFP), its spectral variants and the new red fluorescent protein (DsRed) has opened up new opportunities for the study of the plant secretory system. We are investigating the structural relationship and the transfer of protein between the ER and GA in *Nicotiana* leaves and suspension cultures using fluorescent proteins targeted to the organelles. To target the GA, the trans-membrane domain (signal anchor sequences) of a rat sialyl transferase and a human E1-4 galactosyl transferase were spliced to GFP, YFP or DsRed and to target the ER and GA a chimeric *GFP-aERD2* (the arabidopsis HDEL receptor homologue) was constructed. To study protein flow through the organelles a secretory form of GFP was used.

The Golgi targeted constructs located to numerous small mobile fluorescent bodies in the cytosol which were shown by immunogold labelling to be Golgi. Confocal microscopy of the *aERD2-GFP* construct also showed the probe to be located in the Golgi but also to a low level in the cortical network of ER tubules. Time-lapse confocal microscopy showed that the Golgi were closely associated with and moved over the surface of the ER tubules. The relationship of the GA with the ER was investigated with anti-cytoskeletal agents and the retrograde Golgi-ER pathway was demonstrated with the secretory inhibitor brefeldin A (BFA). Application of BFA in leaves or cells expressing Golgi targeted fluorescent constructs resulted in loss of Golgi fluorescence accompanied by an accumulation of fluorescence in the ER even in the presence of the protein synthesis inhibitor cycloheximide. Removal of BFA resulted in reformation of the Golgi which could be partially inhibited by the expression of mutant Rab 1 GTPase. Photobleaching of individual Golgi stacks revealed a rapid, actin independent, transport of protein between ER and Golgi. BFA treatment inhibited GFP transfer between ER and photobleached Golgi and was also used to inhibit transport of the secretory-GFP to the GA, resulting in a build up of ER fluorescence. This latter transport step was also inhibited by co-expression of mutant tobacco SAR1p, a GTPase predicted to be involved in the early stages of ER to Golgi transport.

Digital Imaging & Image Management in Microscopy

Paul Wetton, Imaging Associates Ltd.

Digital imaging is rapidly becoming accessible in many microscopy applications providing a viable alternative to conventional wet film photography. It offers advantages in terms of portability, ease of duplication, and provides an instant result from the specimen that can be assessed and re-acquired if necessary. In addition the systems used for digital capture allow varying levels of control over the microscope and the acquisition process providing enhanced functionality in terms of image reproducibility, ease of use and automation.
Digital imaging also introduces new options for storage, management and utilisation of the acquired images. Images obtained may represent the culmination of a considerable investment in terms of time and resources and thus have a high intrinsic value. Archiving and cataloguing systems provide the ability to maximise the potential of the acquired images and also secure the images for future reference. These systems now offer many options including; direct image acquisition from within the archiving system, centralised image storage and printing services, and multi-user access to resources.

A Novel Stereological Approach to Quantifying Intracellular Immuno-Gold Labelling Paterens

Terry Mayhew, University of Nottingham.

In Immuno-Electron microscopy, gold particles are used to localise antigens in different intracellular compartments. Their electron density makes them easy to detect, their punctate nature makes them easy to count and the availability of different particle sizes allows multiple labelling to locate different antigens simultaneously. By counting immunogolds, it is possible to quantify labelling distributions between compartments. Usually, this involves estimating labelling densities (LD) which relate the numbers of golds to compartment volumes, profile areas or trace lengths. Here, we present a simpler and more efficient alternative (relative labelling index, RLI) for counting immunogolds in intracellular organelles or on membranes and statistically evaluating the resulting labelling patterns. Testing addresses 2 key questions : (a) is the labelling patern within a group (e.g. control or treated) random ?, and (b) do patterns vary between groups (e.g. control verses treated) ?

The method relies on random sampling of cells, sections and compartments. For organelles, stereolgical test point counting is used to generate an expected distribution of gold particles. For membranes, intersection counts are used. These two variants on the same theme are illustrated using specimen worked examples.

Low Vacuum vs. Low Voltage Applications in the ESEM-FEG Microscope

Stephen M. Habesch, Materials Research Institute, Sheffield Hallam University, Sheffield S1 1WB

There has been a recent resurgence in the use and applications of environmental scanning electron microscopy (ESEM). This reflects the integration of compartmentalised column/chamber vacuum technology and differential pumping systems which are the basic requirements in ESEM, with modern SEM PC-based operating systems. Additionally, modern ESEM systems can be combined with FEG electron sources, significantly increasing the resolution potential and analytical capability in low vacuum gaseous chamber environments. The key difference between ESEM and conventional SEM is the nature of secondary electron (SE) imaging and the detector system. In ESEM, the emitted SEs from a sample surface ionize the chamber gas (H_20vapour, N_2, CO_2, Ar etc.) generating an amplified cascade of "environmental" SEs which are accelerated towards the gaseous secondary electron detector (GSED), capable of generating high resolution images. Additionally, gas ionization generates a stream of positive ions which are attracted to the sample surface and neutralize any excess electronegative charging. ESEM is therefore ideal for the analysis of unstable, insulators without the need for applying conductive coatings. High resolution imaging of wet samples or even liquids (e.g. water-oil mixtures) is now possible on a routine basis. ESEM systems offer considerable analytical potential for insulators including SE and BSE imaging, EDS X-Ray mapping and analysis and EBSD. Novel, more specialised SE techniques reflecting electron diffusion (band-gap imaging) or control of surface charging (charge contrast imaging) can also be utilized . The ESEM chamber can also be used as an environmental "microlab" with the capability for constructing specific temperature, relative humidity, gas pressure and compositional conditions.

However, although an ideal tool for many insulator applications, in some circumstances ESEM analysis is often restricted. High resolution near-surface imaging at lower voltages (<5kV) is particularly difficult in ESEM due to primary beam scattering in the gaseous chamber environment and requires considerable optimization. Comparison of ESEM imaging with low kV imaging in conventional high vacuum FEG microscopes demonstrates significantly improved performance for a range of applications in the high vacuum/low voltage mode. These include fine ceramic components, polymer substrates and nitride/oxide films. Microscopes which combine both ESEM and high vacuum CSEM with good low voltage capabilities can offer the greatest versatility for the surface analysis of non-conducting materials. ESEM mode (low vacuum, high voltage) is preferred where surface environmental conditions need to be preserved or recreated, whereas high vacuum/low voltage is the better option for maximum resolution.

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It is difficult to change the attitudes of a life time, and until 1989 Romania had been a communist country for 50 years. Now, twelve years on, many (but not all) of those in position of authority still have the "old mentality", even in the scientific world, at times actively impeding contact with scientists and institutions in the west. How many of us have to get five different signatures before we can send one work-related fax ? The economy is still in a bad way; even a head of department receives only $100 a month, and the research institutions are similarly hard up.

WE CAN HELP, by sending recent books and runs of journals and equipment ñ even the humble beaker is in short supply, and microscopes (of all sorts, including low power stereo) PH meters, etc are especially welcome, but we can find a good home for almost anything in good working order and with a reasonable chance of obtaining spares and manuals. There are EU grants for multi-national projects, so if you are interested in finding Romanian partners, we may be able to put you in touch with people in the same field; many Romanian Scientists are not able to publish in Western journals, nor have easy access to the Internet. Most of all, SEEC Romania welcomes help with funding; to send a consignment of 200Kg costs over £1000.

Church goers may like to know that Romania will be the focus of the Women's World Day of prayer next March, 2002.

There will be a poster up-date about our work to help scientists in Romania by sending books, journals and equipment and by forming professional links.

Jenny Anne Drewe will be available at the meeting to discuss any aspects of the work of this small group.