

SEMT

Society of Electron Microscope Technology
A celebration of 35 years as a Society



TWO DAY MEETING

Wednesday 27th & Thursday 28th April 2005

at The Open University
Milton Keynes

Prospective members should obtain an application form from the Hon. Secretary:

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The School of Pharmacy, Brunswick Sq. London WC1N 1AX

Tel: +44 (0) 20 7753 5806; **Email:** David.McCarthy@amsl.ulsop.ac.uk

Our web site is: www.semt.org.uk

The annual subscription is currently free

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This year, the **Society of Electron Microscopy Technology** celebrates 35 years of continuous existence, no mean achievement for a small Society. We are marking this by holding a two day meeting over 27th 28th April 2005 at the **Open University, Milton Keynes**, with a variety of topics and speakers, a trade exhibition and a conference dinner.

It is interesting to look back to our humble beginnings, the first meeting taking place at Paddington Technical College in 1970. A group of enthusiasts had been taking part in an AIST course leading to a Diploma in Electron Microscopy and felt the need to set up a forum for the exchange of information and ideas on the techniques and applications of this still fairly new microscopical technique. Among the early members who established the foundations of the Society were Ralph Nunn, Derrick Lovell, Jim Wilkes, Barry Martin, Steve Chapman, Bill Edwards, Pauline Barber, Don Claughner and many others who helped to build it up to become one of the foremost user groups in the country. All aspects of microscopy have been addressed in the meetings, from instrument design and specimen preparation to the latest developments in equipment and techniques.

At first, meetings were monthly in the evenings with just one speaker, but as membership grew, the Society started holding annual one day meetings. Later, additional half day meetings were held where the focus was on one specialist topic, and the evening meetings ceased. Annual visits were also started to various manufacturers of electron microscopes or ancillary equipment, and to research laboratories who could demonstrate new applications of EM technology. These activities provided opportunities for contact between newcomers and the more experienced who could give support and encouragement and share ideas and technical know how. Support from commercial colleagues who bring trade stands to the one day meetings has always been particularly welcome; this provides them with an opportunity to advertise to a keenly interested audience and to obtain customer reaction at first hand.

Within a few years of its establishment, SEMT became affiliated with the Royal Microscopical Society who advertise our meetings in their Proceedings. SEMT was also linked with the British Joint Council for Electron Microscopy. As our needs and circumstances have changed, many different venues have hosted our meetings and we have been very fortunate to have been able to use facilities at St Mary's Hospital, Guy's Hospital, St Bartholomew's Hospital, Bedford College, the Natural History Museum, the Royal Free Hospital, the Imperial Cancer Research Fund, Eastman Dental Hospital, the Royal Veterinary College, Charing Cross Hospital, the Open University and latterly the School of Pharmacy.

Besides the excellence of the scientific papers presented at its meetings, SEMT has a well deserved reputation for always providing bountiful and top quality buffet refreshments. You can see this from the picture right taken at our 21st birthday celebrations. Many friendships, working partnerships and exchanges of ideas take place in a relaxed and informal atmosphere and conference dinners have become very popular in the last few years. A remarkable number of the stalwart characters who moulded the Society in its early days are still with us, and many old friends who have moved or retired keep in touch.

Long ago, we adopted yellow as "our colour", a cheerful, sunny yellow and we used to send out all our notices and communications on yellow paper which showed up well on a crowded notice board! The first logo was devised by Arnold Cleaver, who won the princely sum of £10 as a competition prize! See the left hand design below. This has been modified and modernised (below right) and now needs a white background. But nowadays of course most communication comes by e mail or over the web site (www.semt.org.uk). Some of us, though, still have in our wardrobes a warm yellow sweat shirt adorned with the old logo and a metal badge similarly marked. Past methods of communication have included a diary card showing the dates of all the meetings in one year. In recent years, a wall chart has been an attractive substitute for the diary card and adorns many a laboratory or office; its production is supported by advertisements from our commercial colleagues.



From the very beginning, education has been a prime objective of our meetings. We have several times organised Beginners' Competitions sponsored by the RMS at which short papers are presented by newcomers to the field. We offer special rates for students and encourage poster presentations at the one day meetings. In recent times, we have twice run, in conjunction with Aurion, a course on immunocytochemistry and labelling techniques, and these have proved very successful. We can also boast of the production of a special book, a celebration publication written by Don Claughner to mark the millennium, entitled "A condensed history of the electron microscope". This was available at the very first 2 day meeting the Society had ever held in the year 2000.

And so I conclude this resumé of the activities of SEMT over the last 35 years and invite you to look out for the programme of the next meeting and join us if you possibly can. There is always a variety of topics to interest everyone with an enthusiasm for the latest discoveries and techniques in microscopy and imaging. We hope to have an exhibition of some historic pieces of equipment and pictures of the real old microscopes, so come and enjoy a bit of nostalgia together with cutting edge imaging!

Dr. Jill Lewis SEMT Hon Archivist.





And thoroughly enjoyed ourselves

Day I Programme, 27th April.

- 12:00 p.m. Registration, Buffet Lunch, Trade Exhibition.
 13:50 p.m. Welcome address.
 14:00 p.m. Introduction:
 SEMT Chair, Heather Davies
 14:05 p.m. "Immunolabelling in the study of viral replication".
 Dr. Paul Monaghan; Institute of Animal Health, Pirbright, Surrey.
 14:40 p.m. "ImmunoGold/Silver Staining: Post and pre-embedding applications".
 Peter van dePlas, Aurion, Wageningen, The Netherlands.
 15:15 p.m. Tea, Trade Exhibition
 15:45 p.m. "Viewing metals in animals: the good, the bad, and the deadly"
 Dr. John Morgan, Cardiff University, Wales.
 16:20 p.m. "The Early Days of Electron Microscopy in Leeds."
 Dr. Peter Evennett, Formerly of the Biology Dept., Leeds University.
 17:00 p.m. Wine Reception, Trade Exhibition.
 19:00 p.m. Conference Dinner. An after Dinner speech will be given by Professor Dick Madeley.
- 09:00 a.m. Registration, Tea & Coffee, Trade Exhibition.
 09:30 a.m. "Use of confocal and scanning electron microscopy to study protein dynamics in the inner ear hair cells"
 Dr. Agnieszka Rzadzinska, Sanger Institute, Cambridge.
 10:05 a.m. "EM in Diagnostic Virology" - A Dublin Perspective.
 Patrick Costigan, National Virus Reference Laboratory, University College Dublin.
 10:40 a.m. Coffee, Trade Exhibition.
 11:10 a.m. "Specimen Coating for High Resolution SEM - Problems and Some Solutions"
 Dr. Iolo ap Gwynn, University of Wales, Aberystwyth.
 11:45 a.m. "Searching for WMD" - Micro and Macro Scale, Porton to Baghdad
 Barry Dowsett, Centre for Emergency Preparedness and Response, Porton Down.
 12:20 p.m. Lunch, Trade Exhibition.
 14:00 p.m. "Morphological analysis of the secretory pathway by electron tomography"
 Dr. Koert Burger, Institute of Biomembranes, Utrecht, The Netherlands.
 14:35 p.m. "Serotonin Receptors in the Hippocampus: From Depression to Alzheimer's Passing through Neurogenesis"
 Dr. José Julio Rodríguez Arellano, Biological Sciences, Open University.
 15:10 p.m. "Catching Dust in Space: using analytical scanning electron microscopy to identify remains of micrometeoroids and space debris".
 Anton Kearsley, Natural History Museum, London
 15:45 p.m. Tea, Close of meeting.

TRADE EXHIBITORS :

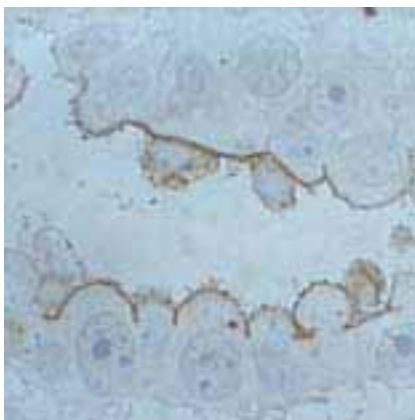
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Immunolabelling in the Study of Viral Replication

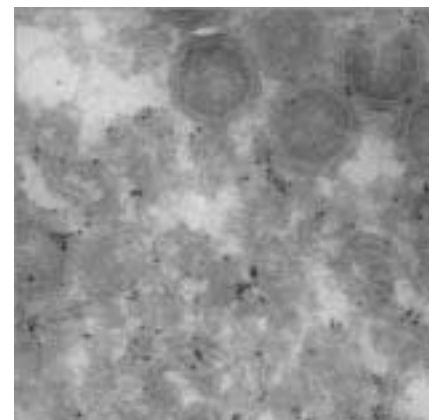
Paul Monaghan, Pippa Hawes, Jenny Simpson, Hannah Cook
Institute for Animal Health, Ash Road, Pirbright, Surrey, GU24 0NF

As this is an anniversary meeting of the SEMT a brief historical look at immunolabelling seems required. Immunolabelling for light microscopy really took hold in the mid 1970's and relied to a large extent on do-it-yourself reagents with detection of antigens by enzyme methods - predominantly horse-radish peroxidase (HRP). Primary antibodies were hard to come by and usually came from biochemical assay systems. For ultrastructural studies, pre-embedding methods were used, but in the late 1970's post-embedding methods tended to use evil reagents to etch or completely remove epoxy resins prior to labelling. Detection was initially with osmium treatment of HRP reaction product. Four main technological steps have been taken since that period; colloidal gold, thawed cryosections, low temperature resins and high pressure freezing and freeze-substitution. These methods along with the massive increase in reagent availability have revolutionised ultrastructural immunolabelling. For a short while, over-enthusiasm with the merits of the confocal microscope led some to claim that localisation of antigens in the electron microscope would be a thing of the past. In fact the two systems are complementary and we have been using a combination of confocal and TEM immunolabelling to study the way in which viruses infect cells. Foot-and-mouth disease virus is a relatively simple virus encoding around 12 proteins and we have been studying virus binding to cell membrane receptors, its interaction with intracellular organelle systems for replication, and the assembly of new virions. African Swine Fever Virus is an altogether more complex virus encoding around 180 proteins and immunolabelling is allowing us to study the assembly of new virions and their subsequent transport to the cell membrane.

From here.....
Epithelial membrane
antigen on the
apical membrane of
breast cancer cells.



to here in just
25 years!
Immunolabelling of
a key protein in the
formation of new
ASFV virions.



Immungold / Silver staining: post and pre-embedding applications

Peter van de Plas, Aurion, Netherlands

Summary

ImmunoGold / Silver Staining: post and pre-embedding applications

Colloidal gold was introduced as a marker in the field of immunocytochemistry in 1971 by Faulk and Taylor. In the 80's the ImmunoGold staining technique was further developed both for microscopic applications and immunoassays. Silver enhancement of conventional immunogold reagents facilitated detection in the light microscope and on blots.

The popularity of the ImmunoGold / Silver staining technique (IGSS) increased since the development and introduction of ultra small immunogold reagents based on a gold particle size smaller than 1nm. Whereas conventional gold reagents can be considered as particles coated with proteins, ultra small gold conjugates can be considered as proteins coated with one or more gold particle(s). The development led to the recognition that not only labeling density but also sensitivity and penetration of immunogold reagents are inversely related to gold particle diameter.

The purpose of any immunocytochemical study is to reveal specific target molecules and assigning them to a particular cell compartment or cellular structure. A compromise has to be found between antigen presentation and antigen accessibility on the one hand and preservation of ultrastructural detail on the other hand.

Post-embedding immunolabeling and ultrathin cryosectioning techniques have proven to be valuable tools in this respect. However these methods are sometimes associated with very limited labeling or technical difficulties.

Pre-embedding immunoelectron microscopy on vibratome or cryostat sections and cell monolayers (cultured cells) is a technique not essentially different from light microscopic immunohistochemistry and is relatively easy. The state-of-the-art of the technique was clearly demonstrated by Yi et al. in 2001 describing a procedure for pre-embedding using ultra small gold conjugates and double immunogold/silver labeling at the ultrastructural level.

The potential of ultra small gold conjugates to label antigens throughout a hydrated specimen provides a unique tool for the development of combined immunoEM-electron tomography approaches.

The Early Days of Electron Microscopy in Leeds

Peter Evennett, Formerly of the Biology Department, University of Leeds
 peter@microscopical.co.uk

This talk will be illustrated with - indeed it will consist principally of - audio and video recordings made in 1982 and 1984, of scientists who pioneered the use of the electron microscope at the University of Leeds from 1943 up to about 1960. Seven RCA EMB microscopes were sent to the UK from the USA in 1943 under the Lend-Lease agreement: goods were supplied to us, and settlement would be made after the end of the war. Leeds was allocated one of these instruments; it went into the care of Professor W. T. Astbury, and was operated by Dr Ron Reed (1920 - 1990), who tells the story of its installation. This EMB was used also by Professor R. D. Preston (1909 - 2000), who, together with Professor Irene Manton (1904 - 1988), later acquired a Philips EM100. Professor Manton used the EM100, and later a Siemens Elmiskop, for her studies on cilia, having observed the '9+2' structure with her ultra-violet microscope before it had been observed by electron microscopy. Siemens microscopes were used also by Dr Jan Sikorski (1913 - 1990) in the Textiles Department, and by Professor Jack Nutting (1924 - 1998) in Metallurgy. The recordings tell the story of the installation and use of these microscopes, in the words of those who were involved.



Dr Ron Reed, Professor Irene Manton, Professor R. D. Preston and Dr Jan Sikorski, photographed on the occasion of the sound recording on 17 February 1982.

Viewing metals in animals: the good, the bad and the deadly

A. John Morgan, Carole Winters, Stephen Stürzenbaum, Peter Kille
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Industrialisation in the UK has created an appreciable legacy of land contamination. The Environment Agency (2002) estimated that between 50,000 and 300,000 hectares of land in England and Wales, spread over $\leq 100,000$ sites, may be classified as contaminated. Most sites are small (< 5 hectares), but between 5,000 and 20,000 of the total may pose unacceptable risk to human and/or environmental health. The main contaminants are organic residues (e.g. hydrocarbons, pesticides, dyes, solvents) and metal/metalloids. Metals will be the focus of this paper.

Current legislation states that contaminated land is defined according to risk assessment principles which, in turn, usually require the establishment of a definite linkage between a source of contamination, an exposure pathway, and a receptor organism. So, merely producing a comprehensive inventory of the concentrations of potentially harmful chemicals in a local soil provides but limited risk-related information. This key point is underscored by considering the relatively (geochemically) 'simple' metalliferous soils associated with disused metal mines. Such soils are spatially heterogeneous, and they are seldom contaminated with a single metal. Metals can interact with each other, and with intrinsic soil constituents (e.g. pH, cation-exchange surfaces of clays and organic acids), such that their availability and potential toxicity to soil-dwelling organisms are modulated (Sample et al., 1998). Some metals are biologically essential ('good'), others are non-essential ('bad'); all metals are toxic ('deadly') above certain metal- and receptor-specific threshold doses.

Earthworms are significant terrestrial receptor organisms for at least three reasons: (i) as ecological engineers they play diverse and fundamental roles in establishing and sustaining soil health and productivity; (ii) their anatomical, physiological and ecological characteristics meld to make them efficient accumulators of several metals; and, (iii) as a consequence of attributes (i) and (ii), they are widely used in ecotoxicology (Spurgeon et al., 2003). It is the net site-specific toxic effects of all the components in a pollution cocktail on a receptor organism that, ultimately, is the priority of environmental managers. Consequently, a large research effort is underway to validate and optimise earthworm response indicators as environmental diagnostic 'tools' analogous to clinical diagnostic protocols; these include molecular genetic biomarkers (Galay-Burgos et al., 2003). Ecological-scale alterations reflect functional responses within individual receptor organisms which, fundamentally, reflect the impact of that proportion of the total accumulated body burdens of potential toxicants free to bioreact with genes and other endogenous molecules. Information about the fate and speciation of metals within receptors such as earthworms is self-evidently important.

This paper describes the use of quantitative electron probe X-ray microanalysis (EPXMA), coupled with anhydrous cryo-preparative procedures and multivariate statistics, to investigate the spatial distributions and ligand-affinities of Ca, Zn (both 'good'), Cd, and Pb (both 'bad') in earthworm (*Dendrodriulus rubidus*) chloragocytes. We found that the metals are discretely compartmentalised within these target cells, with Cd displaying a strong affinity for S-donating ligands, and Ca, Pb and Zn displaying strong preferences for O-donating ligands located in a phosphate-rich

compartment (Fig. 1). These observations illustrate the high spatial resolution capability of EPXMA, as well as its ability to map the co-distributions of detectable elements in 2-dimensional space. EPXMA 'visualises' elements; it offers clues, but is otherwise physically constrained from providing direct element speciation information. This paper shows some of our recent findings on how two complimentary techniques confirm and enhance the EPXMA data. First, X-ray absorption spectroscopy (XAS) (Charnock, 1995) of whole earthworms provides definitive information about metal-ligand co-ordination chemistry: Ca, Pb, and Zn are sequestered by one or more O-donating ligands, although the Pb compound is not pyromorphite; the Cd-S bond distance (2.53Å) suggests a metallothionein-type ligand. Second, immunoperoxidase and immunogold histochemistry, using a polyclonal antibody raised against earthworm metallothionein, provides novel insights into cytological and molecular aspects of Cd detoxification (i.e. how it accumulates without being 'deadly') and trafficking (Stürzenbaum et al., 2001, 2004).

Acknowledgements: We thank the NERC for supporting this work, and all our collaborators (especially John Charnock, Janet Cotter-Howells, John Fry, and Bob Jones) for their various contributions to it.

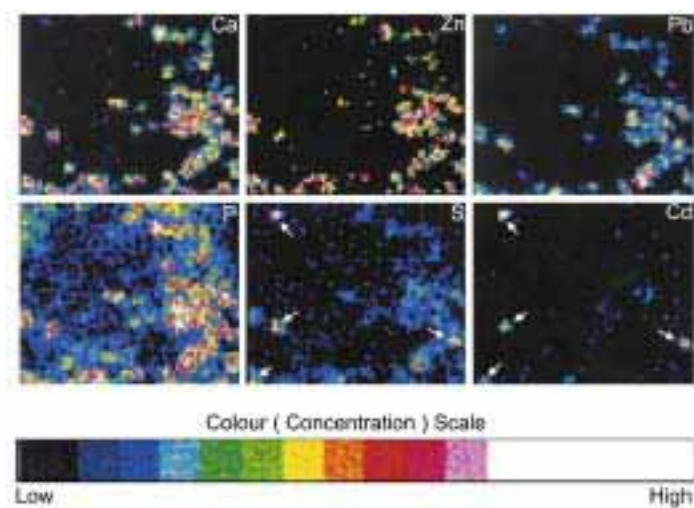


Figure 1: Quantitative X-ray distribution maps for Ca, Pb, Zn, P, Cd, and S in a thin freeze-substituted, resin-embedded, section of a region of chloragogenous tissue from an earthworm, *Dendrodrilus rubidus*, inhabiting a highly contaminated metalliferous soil in South Wales. Note the co-distributions of Ca, Pb, Zn and P on the one hand, and of Cd and S on the other, in separate compartments within the same cells. The colour (concentration) scale is presented as dimensionless because the range of concentrations in each map was different; maximum measured concentrations: Ca (~300 mmol kg⁻¹); Pb (~200 mmol kg⁻¹); Zn (~300 mmol kg⁻¹); P (~100 mmol kg⁻¹); Cd (~200 mmol kg⁻¹); S (~200 mmol kg⁻¹), i.e. white pixels. [Concentrations approx. wet mass values because of the resin content.] The arrows in Cd and S maps highlight the compartment enriched with Cd-S complex. Width of the mapped area ~12_μm.

References

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Use of confocal and scanning electron microscopy to study protein dynamics in the inner ear hair cells

Agnieszka Rzadzinska, Mark Schneider, Bechara Kachar
Sanger Institute, Cambridge

Inner ear hair cell stereocilia are mechanosensitive organelles that detect nanometer scale displacements. The stereocilia are arranged in staircase-like bundles specified precisely with respect to component number and height. Each stereocilium is supported by a rigid paracrystalline array of parallel, uniformly polarized actin filaments that are cross-linked by espin and fimbrin. Mammalian auditory hair cells are terminally differentiated and do not regenerate. While mammalian stereocilia are so orderly structured, so exquisitely sensitive to mechanical vibration and so easily damaged by overstimulation, they are maintained in proper working order for a lifetime. Using transfection of tissue cultured hair cells with beta actin-GFP and espin-GFP fusion proteins and high-resolution confocal microscopy we showed that the stereocilia actin paracrystal undergoes molecular treadmill that maintains the stereocilia length and hair bundle shape in a dynamic steady state. Additionally we investigated the immunolocalization of myosin XVa because mutation in this protein cause deafness associated with very short stereocilia. We observed that myosin XVa caps each stereocilium and its expression levels are correlated with stereocilium length, which may indicate that myosin XVa contributes to self-renewal and dynamic maintenance of the stereocilia. This dynamic view provides new insight into the self-adjusting sensitivity of the hair cell transduction machinery as well as recovery of stereocilia from over-stimulation.

Electron Microscopy in Diagnostic Virology - A Dublin perspective.

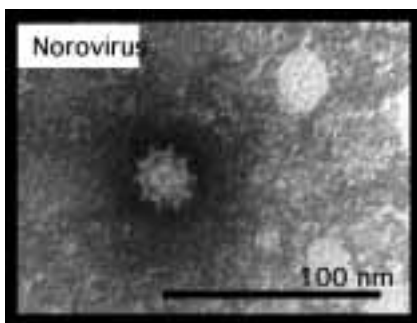
Patrick Costigan. National Virus Reference Laboratory, University College Dublin, Ireland.

The National Virus Reference Laboratory (NVRL) was established in the 1960's and provides a diagnostic virology service to the Republic of Ireland. Since then it has become a CPA accredited laboratory with recognition as a WHO reference centre for Polio, Measles, Rubella and Influenza viruses. From the 1970's Electron Microscopy (EM) has played an integral role in the functioning of the NVRL, and still does so despite the development of molecular diagnostic techniques over the past two decades. The crucial role that EM still plays in diagnostic virology today is clearly demonstrated by its use in the recent global SARS virus outbreak and more locally in the ongoing Norovirus epidemic in Irish Hospitals and other care facilities.

As with most forms of EM, the basic principles have remained relatively stable over the last twenty years. This stability within the field of EM has been advantageous to modern diagnostic virology as it can be relied upon as a gold standard in the detection of active viral infection and as a catch-all technique in more complex clinical cases. As many viral infections clinically manifest quite quickly, rapid and accurate diagnosis has always been essential to determine treatment options. In fact this ability to rapidly and reliably detect and identify viral infection has brought diagnostic EM to a new relevance in the current international political climate with the threat of bio-terrorism and many governments have selected EM as part of their front-line testing protocol in the potential occurrence of a bio-terrorist attack.

In a clinical setting, at the NVRL, EM has proven to be indispensable. A Norovirus epidemic has continued nationwide since 2002 in hospitals and other care facilities in Ireland but is now weakening. This virus is highly infectious, is transmitted through faecal oral route causing diarrhoea and severe projectile vomiting, which can be life threatening to vulnerable patients. However, the main effect is the disruption of ongoing hospital procedures and activities due to the large numbers of patients and staff usually involved in each outbreak. Despite the development of specific detection systems for Norovirus, such as Polymerase Chain Reaction (PCR), they have proven on occasions to be unsuccessful, showing negative results in samples that were subsequently positive by EM. This phenomenon could be explained by the presence of new strains of Norovirus that are not being detected by PCR. Obviously with such a sensitive testing system as PCR the converse will be true and expected sometimes. However, as the interpretation of negative result from EM and PCR are seen differently, the clinician should take some account of this inconsistency. Viral detection using a high-throughput system such as PCR or other molecular techniques which have the potential to be partly automated are optimal as they are less labour intensive than EM, but these techniques benefit greatly by having a parallel EM diagnostic system available.

So as shown in this case, rather than becoming obsolete with the development of molecular diagnostic virology techniques EM has maintained a key role and in similar situations has repeatedly proven its value. The discovery of new viruses and the continued global prevalence of viral infections that are difficult to identify will mean the continued use of EM in the modern viral diagnostic laboratory, essential for patient treatment.



Norovirus negatively stained with Methylamine Tungstate

The Hunt for WMD - Micro and Macro Scale, Porton to Baghdad.

Barry Dowsett. Centre for Emergency Preparedness and Response, Porton Down.

Recent years have seen a heightened awareness of the potential threat posed by bio-terrorism involving the deliberate release of viral and bacterial agents in major centres of population. Electron microscopy has assumed a crucial role as a diagnostic tool for the rapid detection of BW agents in the event of such an incident. This presentation will attempt to illustrate the use of both the transmission and scanning electron microscopes in a 'micro scale' BW diagnostic role and to contrast this with personal experiences and practical aspects of the recent hunt for biological 'Weapons of Mass Destruction' in Iraq.



Specimen Coating for High Resolution SEM - Problems and Some Solutions

Iolo ap Gwynn, The University of Wales Bioimaging Laboratory, Institute of Biological Sciences, The University of Wales, Aberystwyth, Ceredigion, Wales, SY23 3DA

Obtaining optimal high resolution images from an SEM, especially with biological samples, depends not only on suitable specimen preparation but also on choosing the most appropriate set of microscope operating conditions for the specimen being studied and the information required. Assuming that good specimen preservation has been achieved, then a suitable coating must usually be applied. For high resolution secondary electron imaging, a combination of low accelerating voltage (1-3kV) and thin coating of Pt/Pd or Cr usually gives the best results, especially when using a field emission SEM. However, under such conditions exposure of the specimen to the electron beam results in immediate deterioration of the image obtainable - with severe loss of resolution. For some work it is necessary to live with such limitations, and attempt to optimise the coating structure. Some of these problems can be avoided by reverting to backscattered electron imaging. Specimen preparation, and coating in particular, needs to be done in a different way in order to optimise such imaging. Examples are shown of some of the advantages and disadvantages of the different approaches, especially when applied to the study of collagen in the extracellular matrix (Fig 1).

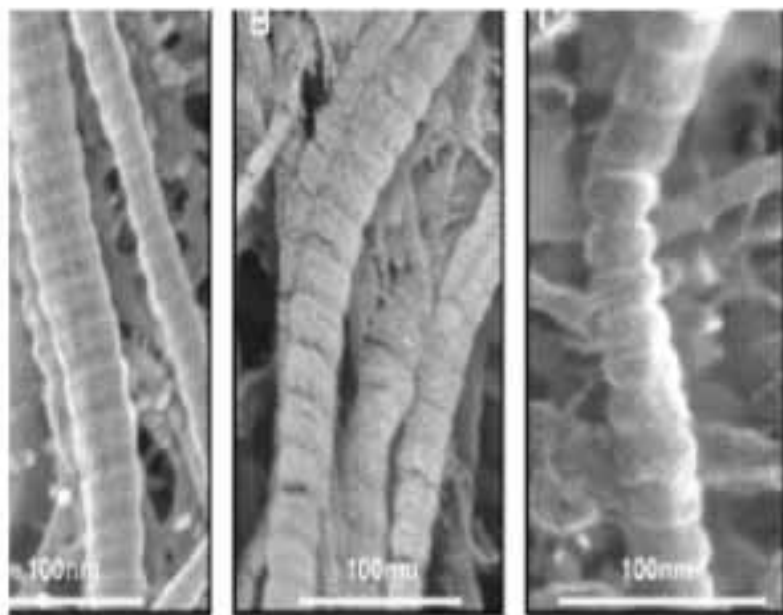


Fig. 1. Field emission SEM images of collagen from rabbit articular cartilage. A. Secondary electron image taken at 1kV accelerating voltage with 4nm Pt/Pd (4:1) coating. B. Backscattered electron image taken at 3kV accelerating voltage, utilising the Hitachi s-4700 ExB filter to eliminate secondary electrons. Specimen coated as for A. C. Backscattered electron image taken at 30kV, using an AuTrata YAG detector, after applying 60nm C to the already applied Pt/Pd coating, as used in A and B.

Analysis of the Secretory Pathway by Electron Tomography

Koert N.J. Burger*, Nuria Jimenez*, Willie J.C. Geerts*, Arie J. Verkleij*, Abraham J. Koster*, Alvar Trucco#, Alexander Mironov#, and Alberto Luini#

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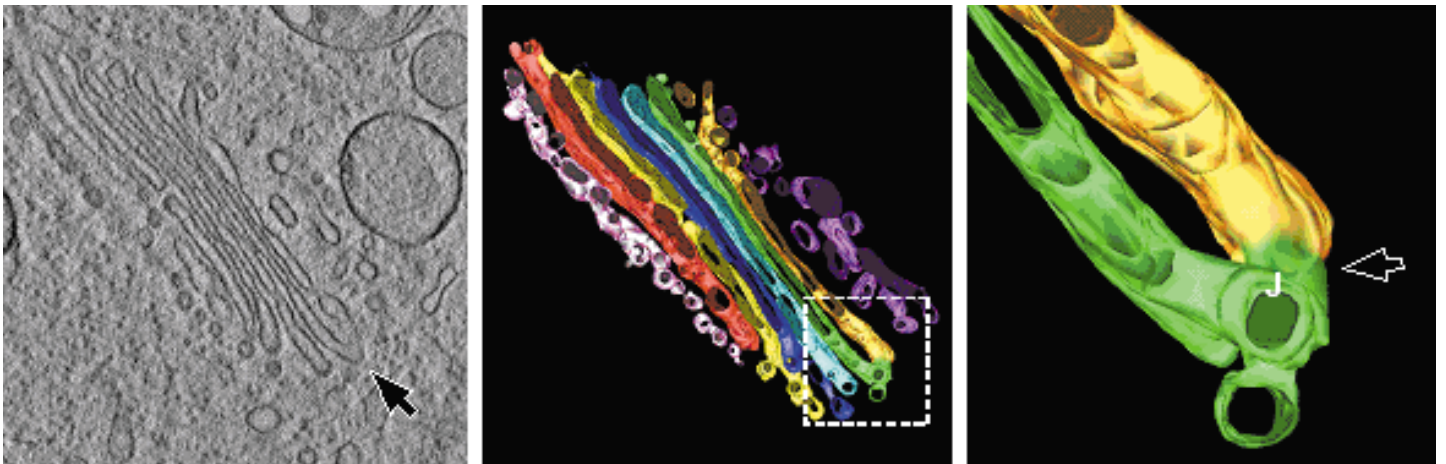
Secretory proteins are synthesized in the endoplasmic reticulum (ER), transported to and through the Golgi stack and ultimately delivered to either the cell surface or the endosomal-lysosomal system. Transport occurs by means of membranous containers, so-called transport carriers. In the classical vesicular transport model, the transport carriers are proposed to be small vesicles (50-100 nm in diameter) that bud from donor and fuse with acceptor organelles (Rothman and Wieland, 1996).

However, live cell imaging has suggested that in several membrane transport steps not small vesicles but larger pleiomorphic transport carriers may represent the main mode of transport (Presley et al., 1997; Toomre et al., 1999). Correlative light and electron microscopy has confirmed the large size and pleiomorphic nature of the transport carriers that mediate Golgi to plasma membrane transport (Polishchuk et al., 2000). Recently we described that proteins move through the Golgi by cisternal maturation i.e. without entering carrier vesicles (Mironov et al., 2001). This finding indicates that the cisternae themselves behave as transport carriers by gradually "maturing" and progressing in the cis-trans direction (Bonfanti et al., 1998).

Thus, a unifying vesicular paradigm for transport carriers is no longer sustainable, and the structure and dynamics of the various carriers that mediate intracellular membrane traffic need to be re-examined using reliable electron microscopical methods and 3-dimensional (3D) image reconstruction. Only 3D-imaging techniques allow one to differentiate between vesicles, tubules, and perforated areas in Golgi cisternae.

The images obtained by conventional transmission electron microscopy (TEM) are mere 2D projections of the 3D ultrastructure contained in the specimens. However, recent developments in electron tomography and computer-based 3D reconstruction now allow a true 3D analysis of cellular ultrastructure with a spatial resolution in the nm-range (Koster et al., 1997; Ziese et al., 2002). Electron tomography involves recording a series of images while tilting a specimen (e.g. a semi-thin plastic-section) over a large angular range ($\pm 70^\circ$) using very small angular tilt increments (e.g. 1°). Subsequently, the images are aligned and a 3D reconstruction is computed via back-projection.

Our recent data on ER-Golgi and intra-Golgi transport in mammalian cells obtained using electron tomography in combination with conventional as well as cryo methods will be discussed (Mironov et al., 2003; Kweon et al., 2004; Trucco et al., 2004).



EM-tomography reveals tubular connections between successive Golgi cisternae within the stack (left, virtual slice extracted from the tomogram; middle, 3D-model of the complete stack; right, detail of the 3D-model indicating a intercisternal connection)

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Serotonin Receptors in the Hippocampus: From Depression to Alzheimer's Passing through Neurogenesis

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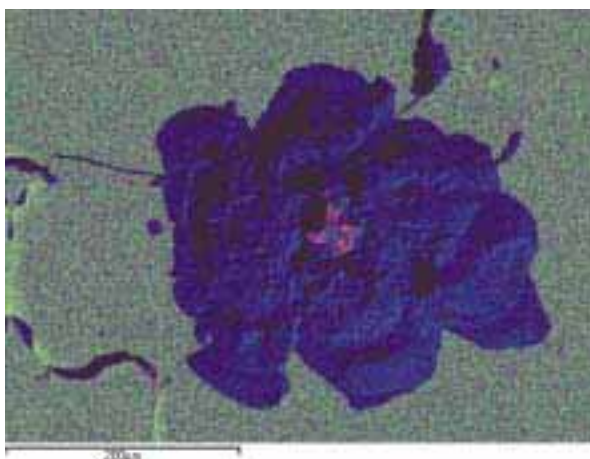
The serotonergic system, which forms part of the trimonoamine modulating system, innervates many brain regions including the hippocampus. Serotonin (5-HT) is implicated in a vast range of neurophysiological processes and behaviours, and intervenes in the etiopathology and treatment of many pathological alterations. Among these disorders the better known are the affective/mood disorders (depression), but 5-HT is being actively implicated as well in the etiology of neurodegenerative diseases and more specifically in Alzheimer's disease. The administration of 5-HT receptor subtype specific antagonists and/or agonists prevents memory impairment and facilitates learning in situations requiring high cognitive demands inducing recovery from impaired cognitive function; but also ameliorate some of the mood disorder symptoms. 5-HT specific receptor activation has also a role in neurotransmitter modulation, stress and neurotrophic processes, including neurogenesis.

Here, we have examined the distribution and subcellular localisation of 5-HT_{1A/1B/2A} receptors and their potential overlap with some of the ionotropic glutamate receptors (NMDAR1/GluR2) in different regions of the rat hippocampus. 5-HT_{1A/1B/2A}-immunoreactivity (IR) was seen mainly within somata, dendrites and dendritic spines. Dendritic labelling was mainly localised to cytoplasmic organelles and specific portions of the plasma membrane. In spines, labelling was associated with synaptic and non-synaptic plasma membranes. These sites were usually distinct from those of NMDAR1/GluR2 which were also seen primarily within dendrites and dendritic spines. Less than 20% of the labelled profiles contained both 5-HT_{1A/1B/2A}-IR and NMDAR1/GluR2-IR. Even if all receptors were present in GABAergic interneurons (less than 8%) the majority of them were present on the main/projection cells of the different hippocampal regions. Our results suggest that somatodendritic 5-HT_{1A/1B/2A} activation may have a role in controlling the input and/or output of pyramidal and (CA1-CA3) and granule cells (GC) being subject to a more subtle modulation involving NMDAR1/GluR1 on more distal dendrites and spines. These findings indicate that in the hippocampus 5-HT_{1A/1B/2A} are strategically targeted to have a major role in cognitive, stress, and trophic processes as well as in the pathological disorders mentioned above.

Catching Dust in Space: using analytical scanning electron microscopy to identify remains of micrometeoroids and space debris

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Space is not empty! Dust particles are released from comets and by collisions between asteroids. These micrometeoroids may in turn collide with spacecraft, for example communications satellites or manned spacecraft. The high velocity of impact (20 to 70 kms-1) results in sudden release of kinetic energy, explosion, and cratering of the spacecraft surface. Whilst the physical damage to the exterior may appear relatively slight, the propagation of high velocity splinters and electrically-conductive plasma into the interior of the craft can prove catastrophic if adequate protection has not been employed. In orbit around the Earth, debris from launch operations may be a greater threat, with fragments of metal and clouds containing trillions of tiny burnt rocket fuel particles moving at 10 kms-1 velocity. To ensure adequate protection without enormous increase in mass, and therefore prohibitive launch costs, it is necessary to achieve a compromise. A very important factor in spacecraft design is knowledge of the number, size, composition and velocity of the particles it may encounter. The most effective way to determine the flux of different types of impacting particles is by examination of spacecraft components returned from orbit. In this presentation I shall concentrate upon the use of analytical scanning electron microscopy in recent imaging and analysis of impact residues on the solar cell arrays of the Hubble Space Telescope HST, returned to Earth by the shuttle orbiter Columbia in 2002. The results not only show a changing pattern of space debris flux since the early 1990's, but have also revealed that the smaller micrometeoroids do not come from the same bodies as the majority of larger meteorites that are collected on Earth. Future particle collections in low Earth orbit may utilise polymer foil blankets to catch grains. I shall also describe our recent work on the analysis of impacts into silica aerogel and onto metallic surfaces, in preparation for the return of the NASA Stardust spacecraft in January 2006, laden with the first samples of cometary dust.



The figure illustrates a typical HST solar cell impact, imaged in X-ray maps for Fluorine (green), Magnesium (red) and Silicon (blue). The magnesium fluoride surface coating has been blown away by the impact, and residue of a micrometeoroid is seen in the strong magnesium enrichment within the central melt pit.