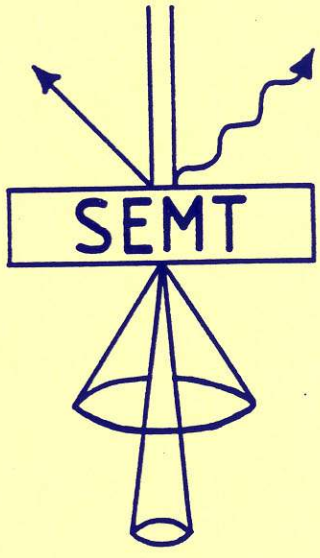
SEMT & PROTRAIN

One Day Meeting, 1994

Friday, 9th December

Institute of Dental Surgery
Gray's Inn Road
London WC1



THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

Prospective members should contact Jill Lewis (the Secretary), 19, Bellfield Avenue, Harrow Weald, Middlesex, HA3 6ST for an application form. The annual subscription is £7. Current committee members are listed below, and are available for further information.

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The SEMT wishes to express their special thanks to:

The Eastman Dental Institute, as hosts, **Stuart Cabeldu Ltd**, as caterers, **Emitech Ltd** for supplying the folders and to the following companies for attending the trade exhibition (in alphabetical order):

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The SEMT hopes that you find the this One Day Meeting interesting and informative, and that you will be able to attend our forthcoming afternoon meetings.

The next SEMT meeting will be *Quantitation: measurement and analysis in microscopy*, and will take place at 1400 on Friday, 22nd February, 1994 at the Eastman Dental Institute, Grays' Inn Road, London, WC1.

WELCOME TO THE ONE DAY MEETING

Friday, 9th December, 1994

Eastman Dental Institute
Gray's Inn Road
London WC1

PROGRAMME

0930	Registration, Trade Exhibition and Coffee
1030	Introduction *Steve Chapman*
1045	The Field Emitter - a Review *Stuart Spiers*
1125	Applications of FESEM to the Field of Polymers *HG Braun (University of Dresden)*
1150	Low Voltage, High Resolution SEM at Low Temperature *Pat Echlin (University of Cambridge)*
1215	Imaging of Lattice Defects in Bulk Materials *Angus Wilkinson (University of Oxford)*
1240	Lunch and Trade Exhibition
1415	Hearing With My Microscope: an FESEM Study of the Inner Ear *David Furness and C.M. Hackeney (Keele University)*
1440	Biological Sectioning by KV *Iolo ap Gwynn (University of Wales)*
1505	Field Emission In-Lens SEM of Nuclear Structure *Terry Allen (University of Manchester)*
1530	Closing Discussion
1540	Tea
1550	SEMT Annual General Meeting

ULTIMATE IMAGING

Abstracts of papers

The Field Emitter - A review

Stuart Spiers (Los Angeles, California)

Applications of FESEM to the field of polymers.

H G Braun (University of Dresden)

The advantage of low voltage scanning EM (LVSEM) for studies on the microstructures of polymers will be discussed from two different points of view.

1) By appropriate conditions surface charging of uncoated non-conducting polymers can be overcome. With modern microscopes, as for example the Gemini 982 (Zeiss), even high resolution imaging of polymer structures such as microfibrils from drawn material becomes possible.

2) The low penetration depth of the electrons at low voltages allows the imaging of thin polymer layers and surface coatings.

The first aspect is especially important for *in situ* deformation studies of polymer materials. The second aspect is of relevance for studies concerning the morphology of thin polymer layers often used as coatings either on organic or inorganic substrates.

The use of LVSEM in both fields offers a wide range of new applications in polymer structure research of basic and industrial relevance. Examples from surface coated fibres, microstructures of drawn polymer films as well as from polymer blends of conducting and non-conducting materials will be shown after discussing the principal design of the Zeiss Gemini SEM.

Low voltage, high resolution SEM at low temperature.

Patrick Echlin (University of Cambridge)

The most sensible approach to the high energy beam microscopy of any hydrated bioorganic material is first, to subject the sample to the least invasive preparative procedures and second, to irradiate it with the minimum amount of energy in order to obtain the maximum amount of structural and chemical information. Low voltage, low temperature digital scanning electron microscopy plays an important part in these processes. The advantages of using low temperatures during sample preparation, examination and analysis are well established, but the advantages of working at low voltages (500 to 5000eV) may be less obvious.

At low beam energies, there is less charging of non-conductors and a significant decrease in beam penetration which will decrease the interactive volume from which any signal is to be collected. The escape depth of secondary electrons is very small and this can be used to great advantage in the study of surfaces. The SE-I escape depth is independent of the primary beam energy and at low beam energy, there is an increase in the SE-I due to the increased fraction of the primary beam generating this signal. However, as the beam energy increases, the sampling depth for the BSE generated SE-II signal increases with a consequent decrease in spatial resolution. The advantages of a smaller interactive volume are offset by an increase in the beam diameter and in some instruments, a decrease in the beam current.

For low resolution (30 nm) images of uncoated material, beam energies of a few hundred volts should suffice, although at very low voltages the beam energy falls below that required for various interactions. For high resolution images it will be necessary to go to higher beam energies (1-5keV) and use frozen dried samples coated with a very thin (1nm) layer of platinum, chromium or tungsten.

Low voltage scanning EM offers some interesting new possibilities for X-ray microanalysis especially when used in combination with the new higher resolution (110eV) germanium ED detectors. At low beam energies, the accuracy and sensitivity of light element analysis improves by a factor of ten although the cross sections for X-ray generation falls off. The X-ray escape path is reduced with a consequent decrease in the absorbency of light element X-ray photons. For light element analysis (Z=6 to 20), there appear to be many advantages in carrying out the analysis between 2-5keV.

Depending on the amount of current in the beam, the ever present problem of radiation damage may intervene. But this too may be ameliorated to some extent by working at low temperatures, low beam currents and using digital integration of fast TV rate scans. The ultimate goal in low voltage, low temperature scanning microscopy must be the 1111 instrument which at a beam energy of 1000eV and a specimen at 100K can give a 10Å spot size with a beam current of 1nA. We are rapidly approaching this goal.

Imaging of lattice defects in bulk materials

Angus J Wilkinson (University of Oxford)

Electron diffraction effects are used routinely in transmission EM for studying the microstructure of crystalline materials. To date, diffraction effects have been less widely exploited in scanning EM, though some powerful techniques are being developed. Two such techniques, electron backscatter diffraction (EBSD) and electron channelling contrast imaging (ECCI) will be described through some applications.

EBSD patterns are essentially Kikuchi patterns which are found in the angular distribution of back scattered electrons (BSEs). EBSD has allowed us to follow the development of preferred orientation (texture) during the abnormal grain growth of a Ni based superalloy from an initial fine grained matrix.

In ECCI the modulation of the BSE intensity caused by local rotations of the crystal lattice is used to form an image of the defect, When field emission SEM is employed ECCI even allows imaging of individual dislocation lines without recourse to thin film microscopy. Various examples of ECCI of defects in semiconductors will be shown.

Hearing with our microscope.

David N Furness and Carole M Hackney

The inner ear contains mechanosensory cells which convert sound or balance stimuli into nerve impulses which are conveyed by the vestibulocochlear nerve to the brain. These cells are known as hair cells because they possess an apical bundle of fine protrusions called stereocilia which are arranged in serried rows. The cells are sensitive to displacements of this bundle; deflections towards the tallest row of stereocilia produce excitation; in the opposite direction, they produce inhibition and orthogonal to this, they have no effect. Excitatory displacements of less than I nm activate a membrane conductance which leads to a flow of cations, predominantly K^+ *in vivo*, into the cell causing depolarization. The cations enter the cell via transduction channels which are thought to each consist of a protein complex containing a pore 0.7nm in diameter. Physiological studies suggest that the channels are operated by a "gating spring" which is connected directly to them.

Observations using electron microscopy have shown that each stereocilium is connected to its neighbour in the row behind by a tiny filament (tip link) which is 2-3 nm thick. The position of the tip link is such that it would be put under tension during excitatory displacements and allowed to relax during inhibitory displacements. This has led to the proposal that the tip links open the transduction channels. However, not all the evidence to date fits this model and other structures around the stereociliary tips may also be important. We are using immunocytochemistry, field emission SEM (in a Hitachi S-4500) and transmission EM to investigate the location of the channels and structures associated with them. Using immunocytochemistry in TEM we have labelled the possible site of the transduction channels. Low voltage FESEM at high resolution has shown new features of the stereocilia associated with this region, and also of the tip links, which may provide new clues as to the operation of the transduction channels.

Biological "sectioning" by kV

Iolo ap Gwynn & Geoff Richards (Univ of Wales, Aberystwyth and
AO/ASIF Res Inst, Davos, Switzerland)

Backscattered electron (BSE) imaging was used to display cellular structures stained with heavy metals within an unstained resin by atomic number contrast in successively deeper layers. Balb/c 3T3 fibroblasts were cultured on either 13mm discs of plastic Thermanox, commercially pure titanium or steel. The cells were fixed, stained and embedded in resin and the disc removed. The resin block containing the cells was sputter coated and examined in a Hitachi 4100 FESEM fitted with a YAG BSE detector. The technique allowed for the direct visualisation of the cell undersurface and immediately overlying areas of cytoplasm through the surrounding embedding resin, with good resolution and contrast to a significant depth of about 2 µm, without the requirement for cutting sections. The fixation protocol was optimised in order to increase heavy metal staining for maximal BSE production. The operation of the microscope was optimised to maximise the number of BSE produced and to minimise the spot size. BSE images were collected over a wide range of accelerating voltages (keV), from low values to high ones to give "sections" of information from increasing depths within the sample. At 3-4keV only structures a very short distance into the material were observed, essentially the areas of cell attachment to the removed substrate. At higher accelerating voltages information on cell morphology, including in particular stress fibres and cell nuclei, where heavy metals were intensely bound, became more evident. The technique allowed stepwise "sectional" information to be acquired. The technique should be useful for studies on cell morphology, cycle and adhesion with greater resolution than can be obtained with any light microscope based system.

Field emission in-lens scanning electron microscopy (FEISEM) in cell biology.

Terry D Allen and Martin W Goldberg (Christie Hospital, Manchester)

Resolution in the SEM is dependent on electron beam diameter and is limited by noise and spherical and chromatic aberrations. 1nm resolution, or better, can be achieved by using a field emission gun which is a thousand times "brighter" than a tungsten filament and can produce a very fine electron beam. Placing the specimen within the magnetic field of the final lens reduces spherical and chromatic aberrations as well as production of SEIII electrons, which are seen as noise. With such an instrument, as with TEM, meaningful resolution of biological structures becomes dependent on specimen preparation. Much thought must be given to sufficiently preserving unsupported, fragile structures such as membranes and protein filaments. Generally, secondary electrons are generated from a thin metal coating. So far, chromium is the metal of choice; it has a grain size smaller than the instrument resolution and sufficient secondary electrons are generated from it. In addition, for immunolabelling studies, the backscatter electron signal of gold particles is easily contrasted from it, making high resolution immunolocalisation possible. FEISEM has been used to study the structure of isolated nuclear envelopes, nuclei, chromosomes and chromatin. These results will be used to demonstrate what can be achieved with FEISEM to obtain new information, even on previously well studied biological structures.

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Ultimate Imaging

Low Voltage, High resolution SEM at Low Temperature
Patrick Echlin
University of Cambridge

Low temperature diminishes but does not prevent deleterious side effects of ionising radiation, and virtually eliminates sample contamination.

If the beam energy is low enough, the beam virtually forms a small cylinder down the column, from which the signal comes and becomes pear-shaped in time. The beam bounces within the pear and emerges some distance from the point of impact as secondary electrons and/or radiation damage.

He hope with the XL to get the timing of the interactive volume. 4 - 5 KeV is needed for microanalysis, with a probe size of 1 - 3 nm. This is for a high resolution image of frozen biological material.

Hearing with my Microscope: an FESEM study of the inner ear
David Furness & C.M. Hackeney
Keele University

The cochlea is the frequency analyser - the Organ of Corti.
He fixes in glut/osmium, then impregnates with OTOTO; he thus avoids using a metal coating. (It is essential to wash well between each stage of the impregnation).
The membrane over the hair cells fortuitously peels back during critical point drying. If OTOTO impregnation has been used, 2 KeV can be used; otherwise 5 KeV is necessary.
An OTOTO specimen can subsequently be embedded and sectioned. The process probably puts a layer on top of the specimen as well as impregnating.

Biological Sectioning by KV
Iolo ap Gwynn
University of wales

A Swiss institute is using metal implants to repair bones; they are looking at the interface.
The cells are (grown ?) on a disk, embedded, cooled and the disk peeled off; then glow-etched and sputter-coated.
At lower accelerating voltage, the contact surface appears rough, and only glows where it touches the substrate at the cell tips. Increasing the emission current to 50 μA, gives a dramatic improvement in picture quality.,
As you change the keV, you change the level at which you get information.
You could get better 3-D information than with a confocal microscope.
It may be easier to interpret the picture as a negative. By image processing, you can get rid of dust, scratches etc.
If sections on a grid are put into a SEM, you can see the parts lying over the grid bars - for counting cells etc.

Field Emission In-Lens SEM of Nuclear Structure

Terry Allen
Univeristy of Manchester

Uses DS 130 --→ 700 series
There is not yet a good standard specimen for SEM at high magnification.
Chromium is good for coating; also if it is difficult to get other metals to stick, use chromium as an intermediate layer; but keep it thin, and have a good vacuum.
Polymerise a few drops of resin in the bottom of an Eppendorf tube; put a 5 mm silicon chip on this, and spin the specimen onto it.
Use the kV appropriate to the specimen and the instrument.
Chromium does not impair the ability of the instrument to pick up the gold labelling.
The Journal of Cell Science is willing to accept papers which include 3-D pictures.

Ultimate Imaging 9-12-94

List of Registrants

Hakeem Al-Dosary	Univ of Wales, Aberystwyth
Terry Allen	Christie Hospital, Manchester
Paul Ansell	Hitachi Scientific Instruments, Wokingham
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Sue Barnes	EMU, Natural History Museum
H G Braun	Univ of Dresden, Germany
Richard Blackburn	Sch. of Environmental Sciences, Univ. of Greenwich
A Booth	ICI Chemicals & Polymers, Runcorn, Cheshire
Alan Boswell	ISS, Manchester
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Tony Brain	King's College, Campden Hill
Heather Davies	Biology Dept., Open University
Ann Dewar	EMU, Royal Brompton Hospital
Barry Dowsett	CAMR, Porton Down, Salisbury
Anne Drewe	Dept. Microbiol., Charing Cross & Westminster Med Sch
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Joanne Hunt	Materials Dept., Open University
Louisa Jones	EMU, Natural History Museum
Chris Jones	EMU, Natural History Museum
Stanley Jones	Microlab Scientific Systems
Lynne Joyce	Agar Scientific Ltd., Stansted, Essex
Mike Kelly	Oral Pathology, London Hospital Medical College
Tania King	Dept Palaeontology, Natural History Museum
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Jill Lewis	EMU, Eastman Dental Institute
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John Warrack	Smith Kline Beecham
Ian Watt	Reading, Berks
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A J Wilson	CCTR, Biol Dept. Univ of York
Naomi Williams	Materials Dept., Open University