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SEMT

Society of Electron Microscope Technology

Affiliated to the Royal Microscopical Society

SCANNING E-M . . . The Future

Friday, 17 May 1991

at

IMPERIAL CANCER RESEARCH FUND
Lincolns Inn Fields, London WC2

PROGRAMME

2.00 Localising cell membrane receptors by High Resolution SEM

Paul Monaghan (Inst of Cancer Research, Sutton, Surrey)

2.35 Examination of uncoated biological material by WETSEM

Paul Taylor (Dept of Palaeontology, Natural History Museum)

3.10 Tea

3.30 Recent developments in biological applications of Scanning Tunnelling Microscopy

Mervyn Miles (Dept of Physics, Bristol University)

4.05 Desktop microscopy – the way ahead

Steve Pearce (Philips Analytical, Cambridge)

4.40 Chairman's Summing up and general discussion

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To the Secretary:

Dr Jill Lewis, Electron Microscope Unit,
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I hope to attend the meeting at ICRF on 17 May 1991

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ABSTRACTS

LOCALISING CELL MEMBRANE RECEPTORS BY HIGH RESOLUTION SEM

Paul Monaghan (Inst of Cancer Research, Sutton, Surrey)

Our work at the Institute of Cancer Research has for many years centred around studies of the control of growth and differentiation in the normal human breast. Whilst it is known that a wide variety of hormones and growth factors are concerned with the growth of the breast epithelium during development (puberty) and differentiation (pregnancy and lactation) the precise cellular targets of many of these agents are unknown.

A number of growth factors interact with their target cell via a cell-surface receptor molecule. As part of a wider investigation into the action of growth factors on the breast, we have used SEM immunolabelling to localise these receptors on defined cell populations.

The receptors are localised with a specific antibody, followed by a second gold-conjugated antibody. The labelled samples are then fixed, dehydrated and critical point dried. Whilst large colloidal gold markers (>30nm) are visible by SE imaging, smaller gold particles are preferable for increased sensitivity, and these are more readily imaged using a back-scattered electron detector. Clearly, surface coating with gold is not appropriate under these conditions, and carbon coating is employed. Unfortunately, the highest antigen detection sensitivity is achieved with a maximum gold particle size of 5nm. Coupled with the lower resolution obtainable using back-scattered electron detectors, these small particles are undetectable in conventional SEM's.

Using a YAG crystal BSE detector fitted to a Hitachi field emission SEM these 5nm gold probes are readily detected. For lower magnifications the size of the gold may be increased by silver enhancement, and such enhanced gold/silver markers may be detected in non-field emission instruments.

Using the methodology described, we have localised specific growth factor receptor molecules to clearly defined regions of the cell membrane.

EXAMINATION OF UNCOATED BIOLOGICAL MATERIAL BY WETSEM

Paul Taylor (Dept. of Palaeontology, Natural History Museum)

Facilities for scanning uncoated specimens have now been in use for over a decade at the Natural History Museum and have been employed extensively in taxonomic and morphological research on living and fossil representatives of several different biological groups. This work was initially carried out using an ISI 60A SEM which was replaced last year by an ISI ABT-55 SEM. Both microscopes are equipped with environmental chambers ('CFAS' and WETSEM respectively) and Robinson detectors. The specimen chamber is differentially pumped to a poorer vacuum (c. 0.1 torr) than the gun and column (c. 0.0001 torr), with the result that the residual air molecules present in the chamber dissipate charge on the specimen through ionization. Back-scattered electrons (BSE's) are collected by the Robinson detector, a fast scintillation detector.

The uncoated system has proved most useful for magnifications less than about 500x, and for relatively flat specimens (as the number of BSEs emitted diminishes rapidly as take-off angle decreases, surfaces at acute angles to the electron beam have poor signal:noise ratios). Edge effects in BSE images are less pronounced than in SE images; the images are not as aesthetically pleasing as SE images but tend to resemble optical micrographs more closely.

The ability to scan uncoated material has many advantages. Foremost among these are that it allows unique and valuable specimens to be studied without alteration. For example, the Museum's SEMs have been used to study many type specimens. These specimens are not only historically important but are crucial to the correct identification and accurate characterization of species. Preparation time is minimised because specimens are simply mounted temporarily on stubs using plasticine, Blu-tack or Leit-C. Curatorial problems arising from the storage of stub-mounted specimens are avoided, and subsequent study of the specimens using optical microscopes can be undertaken without the problems caused by high reflectance from the conducting coat. Furthermore, large specimens (up to 10 cm diameter), difficult to sputter adequately, can be scanned successfully. The high atomic number contrast inherent in the BSE images can also be advantageous when composition varies across the specimen surface.

Uncoated scanning has been used intensively during the study of calcareous skeletons of modern and fossil bryozoans (a phylum of colonial marine invertebrates). Examples of this work will be shown.

Reference:
Taylor, P.D. 1986 Scanning electron microscopy of uncoated fossils. Palaeontology 29 685-690.

RECENT DEVELOPMENTS IN BIOLOGICAL APPLICATIONS OF SCANNING TUNNELLING MICROSCOPY

Mervyn Miles (Dept. of Physics, Bristol University)

The ability of scanning tunnelling microscopy (STM) to give high resolution images in gaseous and even liquid environments has advantages for the study of biomolecules in their native, hydrated state. Images can be obtained without coating or staining, and, in the most favourable cases, atomic scale resolution has been obtained.

The study of (uncoated) non-conducting molecules by STM is in general limited to structures which have a thickness <5nm when deposited on a conducting substrate. For high resolution imaging, molecules must be somehow immobilized on the substrate to prevent movement during scanning. Techniques for immobilization will be described.

Images obtained by STM cannot be interpreted simply in terms of Van der Waals shapes. The electronic properties of the molecule and its interactions with the substrate must be taken into account. Theories of image contrast for STM of biomolecules are being developed.

The advantages offered by other scanning probe microscopes inspired by the STM, in particular the atomic force microscope and the photon scanning tunnelling microscope will be discussed.

DESKTOP MICROSCOPY – THE WAY AHEAD

Steve Pearce (Philips Analytical, Cambridge)

The XL Series of scanning electron microscopes from Philips Analytical introduces the concept of 'desk top microscopy', whereby the array of manual controls used to obtain a wide range of information from conventional systems is replaced by a single personal computer and mouse.

This article explains how the user-friendly mode of operation leads to major time and cost savings in all kinds of imaging and analytical application. In addition, it explains how integration into the rapidly-developing PC environment enables the SEM to become a key link in the information chain extending from data gathering to management decision-making.

Localising cell membrane receptors by High Resolution SEM

Paul Monaghan

Control of proliferation in the breast is by hormones and growth factors. Most cancers come from the epithelial cells lining the lumen. The myoepithelial cells around the ducts contract to expel the milk produced by the epithelium. The stroma contains capillaries etc. The epidermis has growth factor receptors.

He fixes with glutaraldehyde, not with osmium; labelling is almost always with colloidal gold. He coats with carbon, not gold; but it may be difficult to get a good secondary electron image from a carbon coat. With a gold label , the maximum snesitivity is 1 - 5 nm, but the ease of detection is greater than 10 nm. He generally uses a label not bigger than 5 nm; it is possible to use silver anhancement, but this is a "cantankerous" technique and the temperature is vital.

The Back Scatter Detector can be solid state or scintillator (7ag or Robinson), with a perspex light guide for a spark, or a microchannel plate to collect a cascade of electrons (might be best for low kV, but not easy for gold). Solid state detectors are best at 15 kV; the scan rate must be right, too, and the overall geometry. The electron source can be Lanthanum boride or field emission; tungsten is useless!

The Amersham kit is better than other kits, but difficult to use.

Examination of uncoated biological material by WETSEM

Paul Taylor

On uncoated material, he uses an uncharges Robinson high-energy detector for the back-scattered electrons. The secondary-electron detector si highly charge and cannot be sued in a low-vacuum chamber.

The air molecules in teh specimen chamber dissipate the charge automatically. The secondary electron image is more 3-dimensional than the back-scattered.

These specimens are not altered or damaged, because they have no coating. Specimens up to 12 cm diameter can be used; preparation time is minimised. There is no charging even at high kV.

He showed the natural cast of the underside of a Bryozoan, the bio-induration of the zooid.