SOCIETY OF
ELECTRON MICROSCOPE
TECHNOLOGY

ENVIRONMENTAL SEM

2.00 p.m. Wednesday 16 October 1996

EASTMAN DENTAL INSTITUTE
Grays Inn Road
London WC1X 8LD

2.00 **Introduction** - The Chairman

2.05 **Environmental SEM - imaging capabilities and applications**

Chris Gilpin (Sch of Biol Sci, Univ of Manchester)

2.45 **Low vacuum SEM; gold-labelled cells and biomaterials**

Rachel Sammons (Birmingham Dental Hospital)

3.15 **Tea**

3.45 **Dynamic experiments in SEM in materials sciences**

Steven Kitching (Cavendish Laboratory, Cambridge)

4.15 **Field emission SEM; environmental applications**

Dirk van der Wal (Philips Electron Optics)

4.45 **Closing discussion**

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Please reply to the Secretary - Dr Jill Lewis
19 Bellfield Avenue, Harrow, Middx. HA3 6ST
0181 428 4264 (Telephone, Answerphone, Fax)

I hope to be present at the meeting on 16 October 1996

Name .....

Address .....

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ENVIRONMENTAL SEM - ABSTRACTS

Environmental scanning electron microscopy : imaging capabilities and applications

Christopher J Gilpin (EMU, Manchester University)

"Environmental SEM" is often used to describe instruments where the chamber pressure is significantly higher than in conventional SEM. There are, however, two distinct types of such instruments. The main differences between the two types will be highlighted and comparisons drawn.

This presentation will concentrate on the ESEM manufactured by Philips-Electroscan which utilises a secondary electron detector capable of operating at pressures in the range of 1 - 20 Torr (130-2700 Pascals). By use of differential pumping apertures the electron gun and column can be maintained at "normal" vacuum levels of 10^{-5} Torr. A pressure of 10^{-2} Torr exists within the secondary electron detector separated from the specimen chamber by another differential pumping aperture. The specimen chamber pressure is produced by admitting a gas to the chamber under operator control. The gas can be any ionisable gas. When the electron beam strikes the sample secondary electrons are produced. These secondary electrons travel toward the positively biased detector and are amplified in a cascade by gas ionisation. Positively charged gas ions eliminate any surface negative charge on the sample. Water vapour is a commonly used chamber gas. By controlling chamber gas pressure and sample temperature, by means of a Peltier effect stage, condensation and evaporation can be produced. The ability to maintain samples in a fully hydrated state singles out the Electroscan ESEM as a unique instrument. Specific applications for the ESEM will be presented.

Biological samples can clearly benefit from having no preparation, no coating and being kept wet. Studies have been carried out in the author's laboratory on artificial gelatine coated arterial grafts seeded with endothelial cells. Comparisons will be made with conventional SEM and cryo-SEM.

Many previously difficult material science samples can be viewed in the ESEM. Oily samples present no difficulties to either the imaging or vacuum system. Oil-bearing drill core samples have been examined. The oil can be evaporated in the microscope to reveal the untreated structure of the rock pores. This study demonstrates the ability to perform dynamic experiments in the microscope. Indeed with a microinjector, micromanipulator and a hot stage a versatile experimental chamber can be set up.

X-ray microanalysis can be carried out in the ESEM but recent studies by the author and others have clearly demonstrated the adverse effects of a chamber gas. The beam spreads as it passes through the chamber gas causing X-ray data to be collected over a wide area. In addition an X-ray signal is generated by the gas itself. X-ray mapping is a possible method of overcoming this problem.
Clearly the instrument has many varied applications and its potential has yet to be fully explored

Application of a low vacuum scanning electron microscope to the study of biomaterials and mammalian cells

Rachel Sammons (Biomaterials Unit, University of Birmingham School of Dentistry)

The Jeol 5300LV microscope is able to operate in high vacuum or low (reduced) vacuum mode within a range of specimen chamber pressures of 14 to 140 Pascals (0.1 - 1 Torr), achieved by adjustment of the flow of air into the specimen chamber by means of a valve, and the image is formed by back-scattered electrons. Because the number of back-scattered electrons by an element is proportional to its atomic number the clarity of the image is dependent on the chemical composition of the material. One of the advantages of the low vacuum (lv) SEM is that it can be used to examine specimens without the need for lengthy sample preparation and coating with an electroconductive layer of gold or carbon because electrostatic charge on the specimen is dissipated by ions from the air present in the chamber. However, whilst materials of high atomic number do not require any coating, we have found that images of materials composed of relatively low atomic number elements, such as polymers, are greatly improved by gold coating, as shown by examples of vascular catheter materials.

Despite the limitations of the back-scattered electron imaging system of the lv SEM it can usefully be employed to examine uncoated biological specimens, including mammalian tissue. We have used it to investigate growth of rat bone cells in vitro on the calcium phosphate bone substitute material, Interpore. In uncoated specimens cells were visible in lv mode only in cells traversing pores, when they were readily identified by their cell nuclei. Cells not traversing pores were invisible due to the strong back scattered electron signal of the underlying calcium phosphate. Similarly, rat calvarial bone examined uncoated in lv mode showed the bone structure clearly through the overlying layer of osteoblast cells, which were subsequently revealed by gold coating.

The back-scattered electron imaging system of the lv SEM can be exploited to detect immunogold-labelled cells in uncoated specimens. Bone cells producing alkaline phosphatase in rat calvarial periosteum and growing on polystyrene were imaged following silver enhancement to increase the size of the gold particles to approximately 200 nm diameter. In uncoated specimens alkaline-phosphatase positive cells showed up clearly due to the strong signal from the gold/silver label, against a less visible background of non-labelled cells. This technique is potentially useful to study extracellular matrix production on biomaterial surfaces.

These studies demonstrate the complementary use of the lv and high vacuum SEM to study uncoated specimens, material composition, and the behaviour of mammalian cells on biomaterials via the use of immuno-gold labelling. In conclusion, the lv SEM, whilst being a relatively inexpensive microscope, has potentally useful applications in the biomaterials field both for research into cell-material/tissue interactions and in quality control of biomedical devices.

Dynamic experiments in the ESEM in materials science

Steven Kitching (Polymers and Colloids Group, Cavendish Lab. Cambridge)

The ESEM offers many advantages over a conventional SEM. Samples may be imaged in the presence of water vapour or other auxilliary gas such as nitrogen and do not require a conductive coating. By controlling the temperature of the sample and using water vapour as the imaging gas, saturated conditions can be produced (1). It is possible to control the rate of water evaporating from or condensing on the sample and thus the effect of sample hydration or dehydration can be studied in situ.
Cellulose fibres, yarns and fabrics encounter water at numerous stages in their production and use. The swelling of cellulose fibres in the presence of water can affect their size, stiffness and permeability. ESEM can be used to assess the extent of swelling and the reversible nature of the process.

ESEM may also be utilised to study drying processes. For example, it is possible to characterise the microstructure of acrylic lattices during film formation from an aqueous colloidal dispersion to a continuous coating.(2)

The curing hydration properties of cement are of particular importance to the building and oil production industries. The curing of the cement is dependent on a number of factors: the chemical composition of the cement; the curing temperature; the water to cement ratio. The ESEM allows real time in situ observation of the microstructural changes occurring in the early stages of cement hydration. (3)

There are significant difficulties using X-ray microanalysis in the ESEM. Primary beam scattering causes the generation of X-rays from the sample away from the area of interest. However, by carefully controlling the experimental conditions, elemental maps may be obtained tracing the dynamic chemical changes occurring during cement hydration.

1. Cameron, R E and Donald, A M, *J Microscopy*, **173**, 227-237 (1994)
2. Keddie, J L, Meredith, P, Jones, R A L and Donald, A M *Macromolecules*, **28**, 2673-2682 (1995)
3. Meredith, P, Donald A M and Luke K, *J. Materials Science*, **30**, 1921-1930 (1995)

S.E.M.T. 16 October 1996

ENVIRONMENTAL S.E.M.

Environmental SEM - imaging capabilities & applications
Chris Gilpin
University of Manchester

He uses Electroscan = ESEM, a Phillips company.
High pressures may be used - 0.01 - 40 Torr; only the environmental EM can image at 40 Torr. This needs differential pumping, of the specimen chamber against the rest of the microscope.
It uses a Back-scattered detector.
The moisture in the sample will evaporate, so it is best if there is little water there naturally.
In the ESEM, the specimen chamber is at 5 - 10 Torr; there is user-controlled gas admittance to the soecimen chamber, which is large. There is three-stage differential pumping.
The normal beam-specimen interaction produces secondary electrons, which collide with the gas molecules to produce more secondary electrons, i.e. a cascade system; these are attracted to the detector. The gas ions are positive and so cancel the negative charge on the specimen, so there is no need for coating.
Hot stages are possible. A Peltier-controlled stage can be used for cooling.
At 0°C, the vapour pressure of water is 4 - 5 Torr.
The gas used can be water vapour, nitrogen, carbon dioxide, ethanol, argon.
It must be able to ionise, in order to set up the cascade.
A real-time video was shown.

Artificial blood vessels are being developed, for which the lining must be smooth, non-turbulence-forming, non-clotting. Endothelial cells are being grown on a woven substrate.
Hydrated fixed material looks like hydrated unfixed material.

Comparison with cryo microscopy was done on mushroom spores. Control of sublimation is difficult; if gold coating is used, this limits the electron range because you are getting surface imaging. With ESEM, you are working with no coating, low vacuum, and (?) low kV. (with cryo)

Various dynamic studies have been done, e.g. hydration of biomolecules, tearing qualities of packaging polymers. It is possible to get beam damage.
They are looking into the possibility of low kV for imaging surface structure. Obtaining an image at 5kV is hard work, because of the beam getting through the gas. Brendan Griffin in Australia is working down to 300 Volts, but with a modified detector. You are liable to run out of working distance. Above X 1000 there is little surface structure to look at.
In his Institute there are strict regulations about bringing unfixed material into the building.

Low-vacuum SEM: gold-labelled cells & biomaterials
Rachel Sammons
Birmingham Dental Hospital (almost inaudible !!)

She uses a JEOL 5300 LV SEM, which has low vacuum but is not environmental. On biomaterials, e.g. dental specimens.
Electrostatic charge is dissipated by ionisation of the air.
Elemental analysis and electron diffraction are done without coating, at 20 kV.
The BSE detector is directly over the specimen, for a topographical image or a composite image. If it is to the side, you obtain a mixed image.

Bone is covered with cells; uncoated at low voltage, the beam goes through the cells to image the matrix below; at high voltage on coated material, you see the cell surface.

Fixation is in 4% formalin in cacodylate; then dehydration through alcohols to critical point drying. Immuno-gold labelling can be sued.

Dynamic experiments in SEM in materials sciences
Steven Kitching
Cavendish Laboratory, Cambridge

He uses Electroscan E3 with Lanthanum boride filament & Kevex; also Electroscan 2010. With these he can do experiments:
dynamic, e.g. temperature, because the detector is not sensitive to light;
hydration - controlled humidity
mechanical tilting - the material can be stretched, without breaking a coating
XRMA - changes in chemical composition

Possible applications are:
fibre swelling with the addition of water
film formation in paints & lacquers
polymers - beam damage assessment
reservoir technology - filter cakes & drilling muds; cement hydration, as the Al/Fe ratio has to be different from that used for houses; aquatic attack on building materials.

Gypsum controls the setting speed of cement, in its reaction with calcium aluminium salts.

With XRMA, the limitations are "beam skirting", pressure range, and hydration. In "beam skirting", the beam broadens at the base, so that you may receive a signal from several mm from the target area. At 1 Torr, there should be a good signal/noise ratio

Field Emission SEM: environmental applications

Dirk van der Wal
Philips Electron Optics

The XL30 is an ESEM with field emission gun FEG. It can do high resolution imaging of low density materials under environmental conditions. You can get 2 nm resolution at 7 Torr water vapour pressure.

If you can obtain low vacuum, it is an ESEM; if not, it is an environmental SEM. Low vacuum SEM is characterised by the pressure range in the chamber; the detector type; the gas type - air/nitrogen, water vapour/air. With XRMA there is no charging, even on uncoated material. There is a gaseous secondary electron detector. A printed circuit board with detector ring will discriminate SE-1 against SE ii / iii and BSE, and will give a true secondary electron image at low vacuum conditions. It is sensitive to low beam current, and is insensitive to light and heat. It can be cleaned easily, with a toothbrush.

The low-vacuum detector collects only the back-scattered electrons; the resolution depends on the density (atomic weight) of the sample; there is poor resolution on low density material. You obtain pseudo-topography; there is poor signal at low beam current; the detector is sensitive to light & heat.

With zeolites, fine edge detail cna be seen by ESEM, but not by low-vacuum, because you need the secondary electrons.

Beam skirting is a function of the mean gas path length, equivalent to pressure. The skirt affects the signal/noise ratio, and the X-ray point resolution. There is no broadening of the primary beam, leading to high-resolution imaging capabilities.

The FEG beam gives a very stable beam current, down to 3 kV on hydrated material.

ESEM	Low Vacuum
charge-free	charging reduced
high out-gas rate	low rate
true wet-SEM	low dehydration
SE and BSE	BSE only
high resolution on <u>all</u> samples	high resolution only on high density samples
dynamic studies possible	