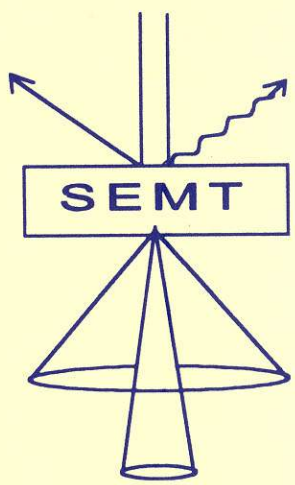
SEMT

ONE DAY MEETING

Wednesday 13 March 1996

Royal Veterinary College
Royal College Street
London NW1



THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

Prospective members should obtain an application form from
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The annual subscription is £7.
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ACKNOWLEDGMENTS

The SEMT wishes to express special thanks to :-

The Royal Veterinary College, London, as hosts
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The next SEMT meeting will be

Environmental SEM

and will take place on Wednesday 16th October at the Eastman Dental Institute at 2.00 p.m..

Our annual excursion is to the CAMR, Porton Down on Wednesday, 5th. June.

WELCOME TO THE ONE DAY MEETING

PROGRAMME

9.30 **Registration**
Coffee, Trade Exhibition

10.20 **Introduction**
Heather Davies (Chairman, SEMT)

10.30 **Tracing connections in the brain**
Paul Bolam (Dept of Pharmacology, University of Oxford)

11.00 **Microfossils - not just a pretty face, pointers to oil and gas**
John Whitaker (Natural History Museum)

11.30 **Electron and X-ray probes in forensic science - shootings and poisonings**
Robin Keeley (Metropolitan Police Forensic Laboratory)

12.00 **Cryo-TEM of frozen hydrated virus particles**
Tim Booth (Institute of Virology, Oxford)

12.30 **LUNCH**
Posters, Trade Exhibition

2.00 Poster presenters in attendance at their work

2.30 **Research on Anglo-Saxon jewellery using an SEM**
Catherine Mortimer (English Heritage, London)

3.00 **The BIG Cryo Debate: the best way to FREEZE**
Paul Monaghan (Institute of Cancer Research, Sutton)
Chris Hawes (Biol. & Molecular Sciences, Oxford Brookes Univ.)
Open discussion

4.00 TEA.

Abstracts of papers

Tracing connections in the brain

Paul Bolam (Dept of Pharmacology, University of Oxford)

In the elucidation of the neuronal networks that underly the functions of the brain a variety of techniques are used that rely on the dynamic properties of neurons. Tracing connections between different regions of the brain (i.e. tract-tracing) is an important and widely used technique. Tract-tracing can be carried out in the anterograde direction, in which the tracer is taken up by the cell bodies and dendrites of neurons and transported to their axon terminals, or in the retrograde direction, in which the tracer is taken up by axon terminals and transported back to the cell body and dendrites. These types of analyses can be carried out at the light microscopic level. However, since neurons within these networks communicate with each other via chemical messengers across synapses, which are beyond the resolution of the light microscope, it is necessary to apply electron microscopic techniques to establish connections between neurons and so elucidate the neuronal networks. In this communication, the application of tract-tracing techniques at the electron microscopic level will be described and will be illustrated by examples from the basal ganglia, the region of the brain involved in Parkinson's disease and Huntingdon's chorea.

Microfossils - not just a pretty face, pointers to oil and gas

John Whitaker (Natural History Museum)

My main specialization is the study of foraminifera (shelled protists) and ostracods (bivalved crustaceans). The are not just aesthetically attractive, they are also a useful tool to the geologist, especially in the search for hydrocarbons.

Planktonic foraminifera first occurred in the Middle Jurassic (175M years ago), have subsequently evolved rapidly and occur extensively in ocean sediments; they are zone fossil *par excellence*. Benthic foraminifera, on the other hand, are used less for dating rocks, more usually for reconstructing palaeoenvironments. The wall structure of a particular group (the agglutinating foraminifera) which construct their tests from sediment, mineral and other exotic grains, has been the subject of a detailed study of mine.

Ostracods have latterly proved particularly useful in determining the changing environments of several Quaternary and Holocene sites in Southern England. Environmental interpretation of the famous Boxgrove archaeological site in Sussex was largely undertaken from ostracod studies.

Electron and X-ray probes in forensic science - shootings and poisonings

Robin Keeley (Metropolitan Police Forensic Laboratory)

Cryo-TEM of frozen hydrated virus particles

Tim Booth (Institute of Virology, Oxford)

Cryo-EM is a valuable technique for investigating virus assembly, and improvements to increase the resolution attainable for biological structures are continually being made.

The main problems with cryo specimen holders for TEM are specimen drift due to expansion caused by temperature instabilities, and mechanical instabilities due to the increased mass of such holders when compared with conventional holders, and the possibility of vibration, or increased susceptibility to acoustic noise, or boiling of the liquid nitrogen coolant.

The Oxford "low mass" nitrogen cooling holder, following a design from Dr Richard Henderson at the MRC in Cambridge can provide 3.4 Å resolution in all directions. The main advantage of this holder over conventional side entry cooling holders is that only the area in the immediate vicinity of the specimen, the specimen cup itself, is kept cold, and thus there are few heat losses which can induce drift through expansion processes which reduce the resolution attainable. Another advantage is improved speed of equilibration to low temperature.

A version of this holder has been specially developed to take advantage of the new Philips Compustage, a cantilever design which has many advantages for low temperature EM, as the lack of a "left ear" completely removes one of the main sources of heat entry and mechanical drifts. The Compustage also gives advantages for 3D reconstruction methods, because of its accuracy of position measurement, storage and recall. The new holder is of a composite structure employing the latest polymers for use at low temperatures.

The holder weighs only 420 g and has a liquid nitrogen hold-time of about 3 hours with a fill volume of only 60 ml. The Dewar design maximises the efficiency of heat conduction and boiling is greatly reduced. Both the low weight and the lack of boiling together with the low cooled mass mean that it is a very stable holder which uses the capabilities of the Compustage to the full. The simple use of an inversion tool can completely stop any minute amount of boiling which may occur for many minutes. Stabilization upon entry into the microscope is frequently achieved in ten minutes. Several design features of the cryo transfer station reduce the ice contamination due to atmospheric water vapour to a minimum, thus protecting the specimen and the microscope vacuum from contamination. Results of graphite spacings and of frozen hydrated virus-like particles using the new holder are presented.

Research on Anglo-Saxon jewellery using an SEM

Catherine Mortimer (English Heritage, London)

Electron microscopy has been used in several different ways to study the surfaces of Anglo-Saxon artefacts when destructive sampling is not possible. In one recent study, topographic backscattered imaging was used to examine silicone rubber peels taken from artefacts decorated with punchmarks, in an attempt to characterize the tools which made the marks. Through this characterization, we hoped to establish how the tools were made and to detemine whether one punch tool was used on more than one artefact. Initial results suggest that only a small number of metal working techniques were used to make the tools but that there were a large number of tools used to decorate artefacts from one cemetery. However, significant amounts of variation in the punchmark images are caused by the way the punches were used and the various types of degradation which artefacts suffered after manufacture. In another study, the surfaces of a gold artefact were analysed using EDX in order to try to establish the number of alloys used in manufacture; the links between the artefact date and its composition were also considered.

The BIG Cryo Debate: the best way to FREEZE

Paul Monaghan (Institute of Cancer Research, Sutton)
Chris Hawes (Biol. & Molecular Sciences, Oxford Brookes Univ.)

Perhaps the first question that must be addressed is whether you really need to freeze. None of the freezing techniques for electron microscopy are simple, cheap and routine. Some are downright difficult and if an alternative is available, it could be worth investigation. The choice of freezing method is determined to a large extent by what you want to freeze and what you want to do with it afterwards. Sample size and the features in the sample that need to be frozen satisfactorily will be important considerations and CryoSEM, frozen hydrated TEM, cryosections, TEM morphology, time resolved TEM, X-ray microanalysis and immunocytochemistry may require different approaches. Of course the only REAL way to freeze things properly is by high pressure freezing, but there will always be "pressure artefacts" and "hexadecene is toxic" muttered darkly by the non-believers. Although the rationale for freezing for both of us is mainly immunocytochemistry, where we really diverge is in the samples that we wish to process and how we go about it. Removal of the frozen water (the word "ice" conjures up thoughts of large crystals which we would rather not see) can be by freeze-substitution or freeze-drying. Which is best ? Is there a better alternative ? Does anyone care ? All will be revealed on the day!

Open discussion

Poster presentations.

In vivo and *in vitro* studies of urinary catheters using the scanning electron microscope

David Patton, Mandy Wooton, Annabelle Hodson, Ben Sale and Nick Ward
(Faculty of Applied Sciences, University of the West of England, Bristol)

Long-term catheterisation of the bladder is considered to be a last resort as a method of managing urinary problems, yet its use is relatively common. In a local study (Kohler-Ockmore, 1992) 48% of 54 patients with long-term catheters presented with catheter encrustation/blockage, the care for which is time-consuming and expensive. *In vivo* encrustations are usually composed of bacteria and crystals of hydroxyapatite and struvite.

This poster describes 3 projects at UWE.

1) Studies of the catheter surface and crystal nucleation on catheters maintained *in vitro* in different simple physiological conditions over varying time periods.

2) An *in vitro* system with artificial urine and controlled pH which has been used to simulate catheter encrustation.

3) A study of the biomaterial/bacterial biofilm interface in clinical samples. This is illustrated with micrographs of early biofilms and of catheter surfaces after removal of encrustations.

Reference

Kohler-Ockmore J (1992) A study of the complications associated with long-term urinary catheterisation and a review of the nursing management specific to this form of treatment. M Phil Thesis, Univ of Wales Coll of Medicine.

Micro-mechanical surface engineering: an alternative to acid etching

B C M Patel, P M Barber, R Laws, S M Dunne & S G Brown
(Eastman Dental Institute, London)

The mechanism underlying retention of plastic restoratives and adhesives is concerned with micro-mechanical interlocking. Fissure sealants, orthodontic brackets, enamel and dentine bonding agents depend entirely on micro-interlocking with the tooth surface micro-structure. It is possible to gain a significant improvement in retention/adhesive properties by creating micro-engineered geometric structures. We have investigated the technique of ultra violet-laser photoablation and image projection with the view to machining banks of 20-100 μm sq (separation of 20-100 μm) geometric structures with a height of 10-50 μm. The surface adaptation of a low viscosity composite resin with the microengineered surface was also examined.

S.E.E.C Romania

Anne Drewe (Microbiology Dept., Charing Cross & Westminster Medical School)

An update on some of our contacts in Romania, their problems, and our efforts to help.

Changes in the numerical density of synapses in the hippocampus of the domestic chick following transient forebrain ischaemia

CH Horner, JY Brown, HA Davies and MG Stewart
(Dept of Biology, Open University, Milton Keynes)

Behavioural changes and neuronal damage following transient cerebral ischaemia (TCI) have been reported in many species including mammals and chicks (Willson et al, 1994). In mammals, the vulnerable CA1 region of the hippocampus demonstrates a dorsal/ventral gradient in the extent of post-ischaemic neuronal damage.

In chicks, it has been shown that TCI causes amnesia only if induced prior to the passive avoidance learning task and neuronal damage has been shown in the chick hippocampus using a silver degeneration technique. It is known that ischaemia leads to a form of excitotoxicity due to elevated glutamate levels with consequent neuronal death. A quantitative study was undertaken to assess the damage at the ultrastructural level by performing a differential count of subtypes of synapses. Hippocampal volume was measured to ensure that any detected alteration in synapse density was not due to volume changes.

The synapses counted were differentiated into subtypes based on the post-synaptic target, namely asymmetric (excitatory) onto dendrite or spine and symmetric (inhibitory) onto dendrite or spine. The counts were performed using a stereological tool, the "disector" (Sterio, 1984). This technique gives an unbiased estimate of the density of synapses per cubic μm; for each disector, only those synapses present in the thickness of one ultrathin section and in a known area are counted. The thickness of a section must also be measured. A formula is applied to these measurements, giving a numerical density of synapses per μm^3.

In this study, there was no change in hippocampal volume indicating no shrinkage following ischaemia. There was a significant reduction in the numerical density of asymmetric synapses in the ischaemic chicks. Thus, pre-training ischaemia impairs long-term memory formation for the avoidance training task in chicks and the hippocampus is a major area of neuronal damage following ischaemia, with a significant loss of synapses.

References

Willson RJ et al; (1994) Transient cerebral ischaemia disrupts performance on a one-trial passive avoidance task in the domestic chick and is associated with neuronal degeneration in the central nervous system. Neurosc **61** 975-981

Sterio DC (1984) The unbiased estimation of number and size of arbitrary particles using the disector. J Microsc (Oxf) **134** 127-131

Diagnosis of transmissible spongiform encephalopathies by electron microscopy

Bill Cooley (Central Veterinary Lab, Weybridge)

The transmissible spongiform encephalopathies (TSE's) or prion diseases are a complex group of chronic, fatal neurodegenerative disorders. The animal TSE's include scrapie and bovine spongiform encephalopathy. The TSE's of man are Creutzfelt-Jakob disease, Gerstmann-Straussler-Scheinker disease and kuru. They are characterized pathologically by vacuolation and astrocytosis, which gives the classical spongiform change within the brain.

However two additional diagnostic criteria for the TSE's, the detection of disease specific fibrils by TEM and the detection of the main constituent of the fibrils, an abnormal protease-resistant neuronal membrane glycoprotein by immunoblotting has also been used.

This poster presentation will demonstrate the involvement of the EM Unit at the Central Veterinary Laboratory in the diagnosis of TSE's by TEM.

Characterization of two bovine enteric calici-like viruses using solid phase immune electron microscopy

AM Dastjerdi, JC Bridger, DR Snodgrass, J Plummer and J Bredl
(Dept Patholology and Infectious Diseases, Royal Vet Coll)

Calici-like viruses (small round structured viruses, SRSVs) were identified as a cause of calf diarrhoea by detection of the viruses in the EM after concentration and purification of faeces (Bridger et al, Infect & Immuno, **43** 133, 1984). Further progress has been hindered by the lack of a quick and sensitive detection method.

We report the use of a solid-phase immune electron microscopy (SPIEM) technique to detect these viruses. Up to 100 particles could be trapped per field at a microscope magnification of 50 K when grids were coated with an optimal dilution of an immunoglobulin concentrate prepared from sera of experimentally infected calves. Examination of daily faecal samples from 6 experimentally infected calves showed that peak virus excretion commonly occurred as clinical signs commenced. Numbers of particles fell sharply on the second day of excretion but virus was detected for at least 3 days.

The antigenic relationship of 2 calici-like viruses, Newbury agent-1 (NA-1) and Newbury agent-2 (NA-2) was examined by SPIEM. Reciprocal cross reactions showed that NA-1 and NA-2 were antigenically distinct. The mean number of particles trapped per field at optimal dilutions of homologous immunoglobulins was 55 for NA-1 and 83 for NA-2 but only occasional particles were seen in heterologous reactions.

The two viruses were purified by CsCl density gradient centrifugation prior to protein PAGE. The buoyant density of NA-1 was 1.34 g/ml and for NA-2 1.35 g/ml. The protein composition of the two viruses is currently being analysed to determine if they have one major capsid protein, a characteristic feature of Caliciviridae.

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Tracing connections in the Brain
Paul Bolam

Tracers were injected into the cell body or near the dendrites, and were found to go to the other end of the nerves. Various tracers were used: HRP, HRP-WGA, kidney bean lectin (Phaseolus), biotinylated compounds. The peroxidase reaction was used, with DAB and with other chromagens.
In HUntingdon's chorea, the brain is shrivelled.
The normal brain has numerous dopamine neurones in the substantia nigra, but in Parkinson's there are very few.
In the basal ganglia, some roots initiate movement, others modify or inhibit it.

Microfossils - not just a pretty face, pointers to oil & gas
John Whitaker

The main micro-fossils of oil-&-gas importance are the Foraminifera, which are shelled Protista; some have only an organic shell, others have shells of calcium carbonate, and the agglutinating forms stick sand grains together to form shells. there are single- and multi-chambered forms.

There is a very good collection at the Natural History Museum, compiled in the last century by a lawyer and a pharmacist. The planktonic foraminifera are the main ones used for dating rocks; these have evolved from benthic forms, and have specialised cytoplasm. They often have symbiotic algae, and many contain droplets of oil; on dying, these form the globigerina ooze. They may be macro- or micro-perforate, the micro holes being about 1½ µ diameter; some have bullae over the apertures.

Some sequences of types can be dated to within 3 million years. Most are 5 - 1 µm (?), but can be up to 1 cm diamter. They are not found on the Continental Shelf or in fresh water, only in deep water.
The Pyramids are built of foraminiferous rocks, a freshwater Nummulitic limestone.
The symbiotic algae allow the organisms to grow bigger, up to 20 cm diameter.

The agglutinating foraminifera, the Ostracods, are important at Boxgrove, where they came from a marine environment, of the Quaternary era.

Electron & X-ray probes in forensic science - shootings & poisonings
Robin Keeley

Orwell - essay - "The Decline of the English Murder"
An example was Graham Young; at the age of 10 he murdered his mother & sister; released at the age of 17, he gained employment with a photographic chemicals company, and murdered several more people with heavy metal poisons.
Heavy metal poisoning is characterised by loss of hair, rigidity, nausea & trembling; in Victorian times it was often by means of arsenic. Probably many cases are now undiagnosed; it is possible to buy some heavy metal poisons abroad readily, e.g. as rodenticides.
A woman had died, diagnosed as "toxaemia of pregnancy", and was cremated, Howver, her hairbrushes had been kept, and arsenic was found 3 - 7 mm from the root. The sensitivity needs to be parts per million.

Shootings: for more than 5 / year, the criminals had hired the gun just for the day! The number of shootings is increasing, and most are drugs-related rather than armed robberies. Many of the weapons used are very old, e.g. service revolvers from the First World War. A sawn-off shot-gun will

produce a lot of noise, light, smoke etc; Shot will penetrate safety glass at 4 yards, with some spread of the shot.
To obtain metal residues from tissue, digest the tissue with enzymes (e.g. in Ariel or Bold); then filter.

Bomb fragments are eroded by the hot gases of the explosion; the object hit will keep some of its structure - this is important for a bomb in an aircraft.
Sophisticated electrical circuitry may be used for the timers; the rest of the bomb is often just lashed up.
A watch with hands rather than digital disply, can still have digital circuitry inside it.

Cryo-TEM of frozen-hydrated virus particles
Tim Booth

He is working on the Reoviridae - Bluetongue virus, reo, rota, colti, cypo (in insects), aquareo (aquatic), various insect-borne plant pathogens; also picorna.
Picorna were the first in which the structure was solved by X-ray crystallography, and is relatively simple
VP3 & VP7 are clear, core-like particles; VP2, 3, 5 & 7 may be fuzzy virus-like particles; they may do self-assembly in baculovirus exporession.

If a thin layer of ice and virus is made across a holey carbon film, VP7 appears as spikes, VP3 as a smooth inner outline. Decoration of the core can be done with VP7 monoclonal antibodies.
Spikeless virions lack polymerase activity.
There is a liposome vector for cytsic fibrosis therapy; the virus aggregates get into the liposomes.
Cryo-EM can resolve 2 Å, but the specimen is radiation-sensitive.

Research on Anglo-Saxon Jewellery using an SEM
Catherine Mortimer

English Heritage does a lot of textile SEM; wood, horn, sheepskin; roots & tubers, pollens; stone tools - wear & working.
EM is used by the departments of Ancient Monuments, Conservators, Technologies.
A red-brown glass of Anglo-Saxon origin contains copper with iron slag in it.
The Ripon Jewel, of gold, garnets and amber, is in the style of southern England.
Jewellery was cast in piece moulds.
There is a wide range of punch-mark styles: ⊙ △ Y ∩ up to 2.5mm across.
The pattern of different punch-mark types indicates different settlements in a large area.
They use a gold-coated rubber peel to study surfaces. (Heather Davies also has used rubber peels.)

The BIG Cryo Debate:

Monaghan
We need an adequate yield of ice-crystal-free sample. If working on micro-organsisms, this is relatively easy because there are so many organisms. A single lump of plant tissue is more difficult.
Cubic ice crystals can be of variable size.
Impact/slamming is good for most tissues; liquid helium is rarely used for cooling, as liquid nitrogen is quite adequate.

If a suspension is sprayed into cryogen, a sludge of beautiful tiny drops is formed; but how to handle them subsequently? Dairy products can be stuck together with cryogen and sectioned.

A grid covered with a thin layer of specimen can be simply plunged into the cryogen.

Jet-freezing (of Hawes) is one of the best ways for plant material and gives good freezing to 2 - 3 cells thick, becuase of the large amounts of sugars in the cells.

All these methods are done on material 10-15 μm thick.

Heterogeneous material requires high-pressure freezing at 30K lb/in^{-2}.

Freeze-fracturing requires fixation, then cryopreservation.

Cryosectioning is difficult; if the material is slammed, you must get the perfect sections within 10 μm.

Freeze-substitution is done at -90°C; amorphous ice recrystallises at -100°C; so it is race between recrystallisiation and getting the water out of the specimen.

High pressure produces super-coolinbg. It is probably not possible to substitute at these pressures. Adjacent cells can be good and bad; this may be because of the physiological states of individual cells, or events during substitution.

Cryosectioning for immuno-cytochemistry is not so difficult, becuase of the sucrose. Without sucrose, it is very hard.

Hawes

Slamming is the best method of cryofixation; he recommends a helium-cooled copper block.

At high pressure, vesicles burst, bundles and filaments re-sort; roots often burst so that resins can get in.

Molecular dry-distillation for controlled low temperature freeze-drying. At liquid nitrogen temperatures, it is possible to freeze-dry, retaining the ions and antigenicity. By slowly raising the temperature, the amorphous ice can be freeze-dried before it changes to hexagonal ice.

Hot osmium vapour can be used as fixative after freeze-drying, then embed in Spurr; the antigenicity is retained!

SEMT Meeting, 13th March 1996

Paul Ansell	Hitachi Scientific Instruments, Finchampstead, Berks.
Sue Barnes	EMU, Dept of Mineralogy, Natural History Museum
Paul Bolam	Dept of Pharmacology, Univ of Oxford
Tim Booth	Inst. of Virology, Oxford
Andrea Boyd	Dept of Oral Medicine, Guy's Hospital, London
Tony Brain	EMU, King's College, LondonW8
John Bredl	EMU, Physiology Dept., Royal Veterinary College
Judith Brock	Oxford Instruments, Eynsham, Oxon
Paul Bunting	Oxford Instruments, Microanalysis Group
Steve Cham	Leica UK, Cambridge
John-Paul Cassella	Dept of Anatomy, St Mary's Hospital Medical School
Jemma Clark	Central Veterinary Lab, Weybridge, Surrey
Bill Clarke	Agar Scientific, Stansted, Essex
Shaun Coles	Burleigh Instruments, Harpenden
Bill Cooley	Central Veterinary Laboratory, New Haw, Weybridge, Surrey
Terry Cooper	Taab Laboratories, Aldermaston, Berks.
Barbara Cozens	Dept of Anatomy, University College, London
Akbar Dastjerdi	Dept of Pathology, Royal Veterinary College
Heather Davies	Biology Dept, Open University, Milton Keynes
Sue Dipple	Sch of Metallurgy & Materials. Univ of Birmingham
Barry Dowsett	CAMR, Porton Down, Wiltshire
Anne Drewe	Dept of Microbiology, Charing Cross Hospital Medical School
Christine Fitzgerald	Emitech, Ashford, Kent
Alan Gray	Dept of Oral Pathology, London Hospital Medical College
Stephen Griffiths	Dept Visual Science, Inst. of Ophthalmology
Chris Hawes	Biol & Mol Sciences, Oxford Brookes University
Roger Hockham	Jeol UK Ltd., Welwyn Garden City
Tania Hopcroft	Histology Lab, Royal Veterinary College
Louisa Jones	EMU, Natural History Museum
Chris Jones	EMU, Dept of Mineralogy, Natural History Museum
Lynne Joyce	Agar Scientific, Stansted, Essex
Robin Keeley	Metropolitan Forensic Science Laboratory
Mike Kelly	Dept of Oral Pathology, London Hospital Medical College
Diane Latawiec	CCTR, Biology, Univ of York
Doug Lock	Princeton Gamma Tech, Peterborough
Jill Lewis	EMU, Eastman Dental Institute
Patricia Lovell	Inst. of Zoology, Regents Park
Allister McBride	Leo Electron Microscopy, Cambridge
David McCarthy	School of Pharmacy, London
Paul Monaghan	Inst of Cancer Research, Sutton
Nicky Mordan	EMU, Eastman Dental Institute
Catherine Mortimer	English Heritage, London
David Patton	Dept of Applied Biology, Univ of West of England, Bristol
Jenny Plummer	BVS, Royal Veterinary College, London
David Robinson	Emitech, Ashford, Kent
Padmini Sarathchandra	Inst of Orthopaedics, Stanmore
Kevin Scudder	Gatan Ltd, Corby
Arthur Smith	Bemax (UK) Ltd. Milton Keynes
Sabrina Sukadi	BVS, Royal Veterinary College
Rosemary Suswillo	Bone Unit, Royal Veterinary College, London
Martin Turner	School of Applied Science, South Bank University
Chris Walker	Philips Analytical, Cambridge
John Whitaker	Natural History Museum, London
Bob Whitenstall	Materials Science Dept., Queen Mary & Westfield College