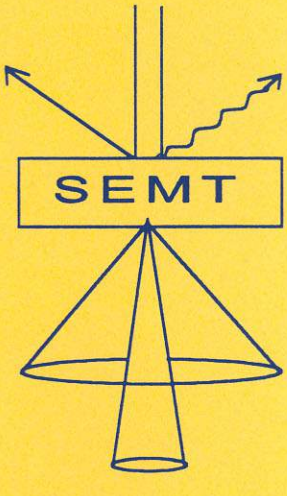
SEMT

ONE DAY MEETING

Wednesday 18 October 1995

Royal Veterinary College
Royal College Street
London NW1



THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

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

Officers	Chair	Sue Barnes
	Secretary	Jill Lewis
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Committee	Richard Blackburn	**Honorary Advisors**	Pauline Barber
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	Heather Davies		Chris Walker
	Anne Drewe		
	Michael Kelly		
	David McCarthy		
	Nicola Mordan		

ACKNOWLEDGMENTS

The SEMT wishes to express special thanks to :-

The Royal Veterinary College, London, as hosts
London's Flying Chef, as caterers
Taab Equipment Ltd. for sponsorship and for supplying the folders
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The next SEMT meeting will be

"The cutting edge: contributions from members"

and will take place on Wednesday 6th December. This will be followed by the AGM.
If you would like to provide a 10 minute oral presentation, please contact the Secretary not later than 1st November.

WELCOME TO THE ONE DAY MEETING

PROGRAMME

9.30 **Registration**
Coffee, Trade Exhibition

10.20 **Introduction**
Sue Barnes (Chairman, SEMT)

10.30 **"There's a wonderful view from the top"- using 3-dimensional SEM**
Bernard Breton (Cambridge University)

11.00 **The private life of a biofilm**
Pauline Barber (Eastman Dental Institute)

11.30 **80 years of electron probe microanalysis**
Graham Cliff (University of Manchester)

12.00 **E M visualization of mycorrhizal fungal slime**
Hilary Denny (Open University)

12.30 **LUNCH**
Posters, Trade Exhibition

2.00 Poster presenters in attendance at their work

2.30 **Meteorites and micrometeorites down the microscope**
Monica Grady (Natural History Museum)

3.00 **Macrophage apoptosis *in vitro* and in human atherosclerosis**
Laszlo Hegyi (Cambridge University)

3.30 **TEM section-style images from an SEM**
Geoff Richards (ASIF Research Institute, Davos, Switzerland)

4.00 Final discussion and TEA.

Abstracts of papers

"There's a wonderful view from the top"- using 3-dimensional SEM

Bernard Breton (Cambridge University)

This talk will present modern techniques for stereo imaging in the scanning electron microscope, with a brief retrospective on older methods. The current generation of computer-based SEM's enable real-time dynamic stereo to be acheived and provide a new source of valuable information for every microscopist. Video footage of real-time static and dynamic stereo will be shown to demonstrate the results obtainable by this technique. The quality of stereo imaging, like most aspects of microscopy, depends on the skill of the microscopist, and so the talk will conclude with some helpful advice regarding how to optimize instrument parameters in order to gain maximum benefit from this technique.

The private life of a biofilm

Pauline Barber (Eastman Dental Institute)

A bacterial biofilm is an accumulation of bacteria and their products on a surface. In some instances, biofilms are considered to be advantageous. In others, however, such as infection, biocorrosion and biofouling, biofilms are deleterious and this has led to an explosion of interest in biofilms in recent years. In their natural environment, bacterial cells are usually surrounded by a highly complex hydrated layer known as the glycocalyx. This comprises the matrix of the biofilm, keeps the integrity of the film together, allows its adherence to a surface and probably acts as a barrier controlling the passage of molecules. For this reason, we need to increase our knowledge of biofilm characteristics. Cultural and biochemical studies have provided much information on modes of growth and bacterial responses to controlled changes in their environment. Various EM studies using both old and new techniques have yielded information on biofilm morphology, modes of growth and development and mechanisms of adherence.

The human dental plaque is one of the most common naturally occurring biofilms and therefore an obvious *in vivo* study model. This brief talk will therefore be in two halves. Firstly, laboratory and electron microscopical techniques for biofilm study will be discussed. Secondly, recent research on human dental plaque will be presented which demonstrates how cultural techniques, biochemistry, molecular biology and electron microscopy can be successfully combined to provide evidence on the virulent properties of a gram negative constituent of human dental plaque.

80 years of electron probe microanalysis

Graham Cliff (Materials Science Centre, University of Manchester/UMIST)

In this centenary year of the discovery of X-radiation it is perhaps fitting to reflect on the fact that it is now 80 years since the death of Howard Moseley. To quote Dr S Butler "H.G.J.Moseley was probably Rutherford's most brilliant associate!" Whilst most scientists know the work of Rutherford and many people are aware of the device developed by Geiger (the Geiger counter) few are as well aware of their colleague Howard Moseley. His work on electron-induced X-ray emission from bulk metal targets laid the foundation for our understanding of atomic number. It gave Niels Bohr, whilst he was working in Manchester with Rutherford, the confidence to justify his quantised ideas about the atom and, as Moseley himself pointed out, offered the prospect of a non-destructive way of chemical analysis.

This means of analysis is now a routine analytical facility on most scanning electron microscopes, whilst electron-induced X-ray analysis on the transmission electron microscope has developed to the point where spatial resolution in analysis is now on a scale of nanometres and detection limits are approaching atomic levels.The development of the technology behind the technique of X-ray microanalysis has a fascinating history and this presentation will try to inform as well as entertain.

E M visualization of mycorrhizal fungal slime

Hilary Denny and Heather Davies (Open University)

In recent years considerable interest has centred on the role of the extrahyphal slime produced by filamentous fungi in the adsorption of potentially toxic metal ions from the surrounding medium. Ectomycorrhizal fungi have been found to ameliorate zinc toxicity to the two British species of birch, Betula spp. and to ericaceous mycorrhizas (comprising Calluna vulgaris and the fungus Hymenoscyphus ericae) exposed to Cu and Zn toxicity. Results of X-ray microanalysis showed that the metals may be adsorbed to electronegative sites in the region of the hyphal cell walls and there is circumstantial evidence to suggest that the extra-hyphal polysaccharide slime may play an important role in this respect. The aim of the research was to try and clarify the role played by extra-hyphal slime in the detoxification mechanism.

The most commonly used method for preserving and revealing extra-hyphal slime on filamentous fungi for electron microscopy, has been to use uranyl acetate as a post-fixation step. However, this method has some disadvantages. Uranyl acetate binds to protein moieties in the slime yet extra-hyphal slime is glycoproteinaceous, and as such the number of protein moieties in it is greatly exceeded by the number of carbohydrate moieties. Consequently, uranyl acetate tends to produce a relatively low intensity of staining of the slime, while the cytoplasmic contents of the hyphae are overstained. A method of staining slime which combines a high degree of slime preservation with optimal intensity of staining of both slime and hyphal contents is needed.

The method that has been developed is an adaptation of a method used to stain the extracellular bacterial glycocalyx. It employs ruthenium red in conjunction with osmium tetroxide instead of uranyl acetate during the fixation steps. The ruthenium red reaction cannot be completely categorized, but in conjunction with osmium tetroxide it binds to proteins, glycogen, polar lipids and acidic oligosaccharides, unlike uranyl acetate which reacts solely with protein moieties. We present the results of a study to compare the preservation of the slime layer of these isolates using the uranyl acetate and ruthenium red methods of specimen preparation. The method reported here appears to satisfy the criteria listed above, and it has been used to demonstrate the presence of a previously unrecognised layer of loosely adherent slime, which is found associated with certain fungal isolates. It is thought that this may be a principle site of metal ion sequestration in cases of metal toxicity. These findings may also be of importance in clarifying the role of extra-hyphal slime in recognition events such as the establishment of both mutualistic and pathogenic interactions between fungi and higher plants.

Meteorites and micrometeorites down the microscope

Monica Grady (Natural History Museum)

Meteorites are natural objects that survive their fall to earth from space. They fall essentially at random over the earth's surface, and the inner portion is cold when they land; only the outermost layer melts, due to frictional heating during passage through the atmosphere. Almost all meteorites come from the Asteroid Belt, orbiting the sun between Mars and Jupiter. When the Solar System formed, approximately 4560 million years ago, asteroids also aggregated along with the sun and planets. The earth is an active planet - all the original material which went into its formation has been recycled through plate movement ("plate tectonics"). Thus meteorites remain our only opportunity for direct study of the primitive material from which our solar system was built. Meteorites vary in size (from enormous crater-forming bodies several kilometres in diameter to micron sized dust) and composition (from iron meteorites, not unlike stainless steel, to stony material), close to terrestrial basalts in composition.

It has been calculated that approximately 50,000 tonnes of extraterrestrial material falls to the Earth each year. Over 95% of this arrives as cosmic dust, or micrometeorites: micron-sized particles which settle slowly through the Earth's atmosphere, much of it without melting . Micrometeorites are collected either in the stratosphere, by high flying aircraft fitted with silicone oil-coated plates, or in Antarctica, by melting of Antarctic ice. The size of these particles ensures that the only effective methods of determining their mineralogy, chemistry and structure is through electron microscopy (SEM and TEM).

Larger meteorites, collected by more traditional methods are initially characterised by optical microscopy. In order to learn about the texture and compositional relationships between millmetre-sized components within meteorites (called chondrules, once molten droplets of silicate), thin sections are prepared from the rocks and analysed by SEM. However, also buried within the fabric of meteorites are sub-micron sized interstellar diamonds, produced in the outflowing wind of neighbouring stars, and mixed into the dust cloud that eventually became our solar system. These nanometre-sized diamonds have been isolated from meteorites by dissolving the bulk of the rock in oxidising acids. This allows a TEM mount to be made of the resulting residue, from which we have learnt the structure of nanodiamonds, and thus inferred possible formation mechanism.

It is therefore possible to use electron microscopy as a tool to trace the processes that have shaped the evolution of the solar system, through study of components within meteorites and micrometeorites.

Macrophage apoptosis *in vitro* and in human atherosclerosis

Laszlo Hegyi, Jeremy N Skepper*, Simon J Hardwick, Wei Zhang, Nat R B Cary@ and Malcolm J Mitchinson.
Dept of Pathology, University of Cambridge, *Multi-imaging Centre, Dept of Anatomy, University of Cambridge, @Dept of Pathology, Papworth Hospital, Cambridge, UK.

It has been known for many years, from comparative studies of atherosclerosis between underdeveloped and industrialised societies that the pattern of lesions differs between the two. The underdeveloped populations develop fatty streaks (early lesions) just as much as the developed ones, but they have a much diminished incidence of advanced lesions with basal areas of dead tissue (lipid core) explaining the low incidence of the potentially fatal complications of the disease, such as myocardial and cerebral infarction, in these populations.

In the formation of the lipid core during atherogenesis most consider that a major source of the material is the death of macrophage foam cells. In support of this postulate the core contains large amounts of two components which in earlier lesions are only found within macrophages - ceroid pigment - and a monocyte/macrophage-associated antigen reacting with CD68/PG-M1 antibody. The cause of the cell death in human atherogenesis is still not known. Oxidised low density lipoprotein (LDL) is a potential candidate. Certain oxidation products of cholesterol which are known to be toxic are found in lesions during human atherogenesis and lipophilic antioxidants such as DL-α-tocopherol and probucol have proved to be protective against this toxicity *in vitro*. The possible beneficial effects of antioxidants in humans have been described by epidemiological and clinical studies.

Human monocyte-macrophages exposed to oxidised LDL undergo apoptosis *in vitro*. Therefore we sought evidence for apoptosis in human atherosclerotic lesions.

Selected coronary artery lesions from hearts removed at orthotopic heart transplantation were examined by light and electron microscopy. Several methods were used including fluorescence staining of DNA, immunocytochemistry and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labelling (TUNEL) method at the LM and EM level. We modified the previously described TUNEL method, the highest dilutions of the TdT enzyme and the best incubation time have been determined on adjacent sections resulting in optimal staining with the least amount of background. We also modified the TUNEL method for EM by using colloidal gold to demonstrate the incorporation of biotinylated dUTP.

We found that apoptosis occurs in human atherosclerotic lesions in addition to necrosis. It has been proposed that the severity of insult to cells determines the route to cell death. A mild insult may cause apoptosis and a severe insult can lead to necrosis. Indeed cell death is most prevalent at the boundaries of the largely acellular core of the lesion suggesting that the potential damage to the cells is greatest in this area. At present, the ratio of apoptosis to necrosis within the lesion is unknown.

In view of the toxic effect of oxidised LDL on macrophages *in vitro* and the protective nature of antioxidants *in vivo* we propose that oxidised LDL-induced cell death, including apoptosis, may have a role in atherogenesis. The exact mechanisms involved in atherogenesis merit further investigation.

TEM section-style images from an SEM

Geoff Richards (ASIF Research Institute, Davos, Switzerland)

Backscattered electron (BSE) imaging, providing atomic number related contrast, is used here to display heavy metal stained structures within various biological specimens within resin blocks. Samples are fixed, stained and embedded in resin as for TEM. The resin blocks are trimmed to centre the specimens in a trapezoidal face of up to 5 mm^2 and their sides painted with conductive silver paint, leaving the face uncovered. Blocks are then sputter coated with 6-8 nm of aluminium which provides a high specimen contrast in BSE mode. Samples are then examined in a field emission scanning EM operated at a high emission current of 50 µA. Both the fixation protocol and microscope operating parameters are optimised to maximise the number of BSE available from the smallest probe. The technique allows visualisation of biological samples embedded in resin at a medium resolution with good contrast, without the requirement for cutting sections, and avoiding the difficulty of grid bars obscuring areas of interest. The images obtained are two dimensional providing averaged information on the internal structure of the specimen in relation to the depth from which the BSE would be expected to be emitted. The technique can also be used for routine preselection of areas of interest within a sample face before final trimming for ultramicrotomy and production of ultrathin sections for higher resolution TEM study.

The high emission current BSE production technique can also be used for observation and immunogold labelling of focal adhesion sites from the upper and undersurfaces of cultured fibroblasts, viewing cells through an embedding resin to display areas of cell contact with à substrate.

Poster presentations.

Localisation of the trypanocidal drug Samorin (isometamidium chloride) in a sensitive *Trypanosoma congolense* clone by fluorescence, immuno-EM and autoradiography.

Clive Wells (International Livestock Research Institute, Kenya)

Samorin is an trypanocidal drug that is widely used both to prevent trypanosome infections in livestock and to cure animals already infected with this deadly parasite. Farmers throughout the world's tropical regions have relied extensively on this compound for the last 30 years to protect their stock from potentially fatal infections transmitted by the bite of infected flies. Unfortunately, evidence of parasite populations developing high degrees of rersistance to Samorin is increasing. To prevent this situation from becoming any worse, it was critically important to discover how the parasites resist the drug.

Previous research suggested that parasites killed by Samorin take up large amounts of the drug, whereas resistant populations take up only very small (sub-lethal) amounts. To determine how the parasite resists the drug it was necessary to discover how the drug is taken up by the trypanosome and where the drug is targeted once it gets inside the parasite. We definitively revealed the target within the parasite as the kinetoplast. Moreoer, the results gave important clues as to how the drug enters the cell. With this information - plus knowledge being disclosed in on-going experiments on the mechanisms employed by resistant parasites to avoid drug action - we can begin to research ways of bypassing the mechanisms of drug resistance. In this way we hope to extend significantly the lifespan of this important drug.

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.

Picro-Hibiscin: a stain of value for degenerated muscle fibres.

Fathi M Gowali (Histology Dept., Charing Cross & Westminster Medical School)

Using the Hibiscin stain or its modification Picro-Hibiscin, collagen fibres were successfully demonstrated. They stained bright red and appeared blue or blue-green under polarised light. Also nuclear chromatin, muscle fibres and collagen fibres were differentially stained. The results were not only comparable with the Haematoxylin-van Gieson stain but had significant advantages, including the demonstration of fine collagen fibres and greater permanence. In the work reported here, Picro-Hibiscin was found to be of great value when the stain was applied to human dystrophic muscle, myocardial infarction and necrotic skeletal muscle fibres in the mdx mouse model.

The tissues were fixed in neutral buffered formalin and routinely processed. Sections were oxidised with periodic acid and incubated in Picro-Hibiscin solution followed by iron-alum treatment. They were then mordanted with phosphotungstic acid and incubated again in Picro-Hibiscin solution.

Normal muscle fibres were stained yellow and necrotic muscle fibres as well as some macrophages were stained dull pink, while myotubes were grey. In addition the nuclear chromatin was blue-black and collagen fibres including the endomysium were bright red.

Light green safranin: a staining method for malarial parasites in paraffin sections.

F.M.Gowali and N.Francis (Histology Dept., Charing Cross & Westminster Medical School)

This study was performed to facilitate the identification of malarial infected red cells in formalin fixed tissue sections derived from placental and other tissues, where *P. falciparum* infection is common.

In Haematoxylin and Eosin stained sections, the diagnosis of malarial infection is based mainly on the presence of characteristic blackish-brown haemozoin pigment in red cells and macrophages. Althougjh the parasites may be visualised, this is often dependent upon successful prior differential picric acid bleaching to remove formalin pigment, but this may also remove the diagnostic haemozoin. In addition the parasites are often poorly differentiated as pale bluish grey against the orange-red erythrocytes.

This work describes a new method based on the application of a light green safranin combination which allows rapid differential staining of red cells (green) and the parasites (orange-red to magenta) and allows the parasites to be seen when pigment is present. The simplicity and stability of the staining method described makes it a useful operation for routine use in the diagnosis of malarial infection in tissue specimens.

Pathological findings associated with trigeminal neuralgia due to vascular compression.

Terry Gradidge (Dept. Neuropathology, Frenchay Hospital, Bristol)

Vascular compression of the trigeminal nerve root accounts for more than 80% of intractable cases of trigeminal neuralgia but the pathogenesis is still debated. We report the ultrastructural changes in the trigeminal nerve root, from a patient with trigeminal neuralgia, at the point of compression by a large medially placed petrosal vein, and compare these with findings in six cases of trigeminal neuralgia not related to vascular compression. Vascular compression of the nerve root was associated with focal loss of myelin and close apposition of the demyelinated axons with few intervening astrocytic processes. No inflammatory cells were seen. Immunoelectron microscopy for GFAP confirmed that astrocyte processes were largely confined to the periphery of the lesion. Of the other six rhizotomy specimens, only one, from a patient with multiple schlerosis, showed demyelination, with intervening astrocyte processes and perivascular lymphocytes and lipid-laden macrophages. Our findings support the hypothesis that ephaptic transmission plays a role in the pathogensis of trigeminal neuralgia related to vascular compression.

SEM Investigation of potential desensitizing agents in the dentine disc model.

T.Y.Y.Ling, D.G.Gillam, P.M.Barber, N.J.Mordan and J.Critchell*
(Eastman Dental Institute)(*Jeol UK Ltd)

Currently the most accepted mechanism of intradental nerve activation associated with dentine sensitivity appears to be hydrodynamic in nature. The concept of tubule occlusion as a method of dentine desensitization is a logical conclusion from the hydrodynamic theory. The dentine disc model has been previously used to demonstrate the potential occluding properties of selected desensitizing agents. Dentine discs were prepared by the method of Mordan, Barber and Gillam. The surface deposit on the dentine disc after two minutes of brushing and upon rotation with saliva supernatant for six hours was examined in a Jeol 6300 Winsem. X-ray microanalysis was undertaken to characterise the nature of the deposit using a Linkisis cell energy dispersive analyser. Of the desenitizing agents examined, Sensodyne sealant (Ferric oxalate) produced initial crystal-like structures occluding almost all the tubule orifices. These results were superior to Butler Protect (Potassium oxalate). Silica and calcium carbonate-based components of various toothpastes were observed both on the surface and within the tubules indicating a degree of therapeutic potential for these two components. Tubule occluding properties could not be demonstrated from the other agents under test (potassium salts, stannous fluoride and strontium chloride), the surface deposits of which were readily removed by rinsing. The results of this study support previous findings with regard to the nature of the deposit on dentine. Toothpaste abrasive/filler components, in particular calcium carbonate-based toothpastes (MacLeans Freshmint) appear to have potential tubule-occluding properties as demonstrated in this study. Ferric oxalate would also appear to have potential as a tubule occluding agent.

S.E.M.T. One-Day Meeting 18 October 1995

"There's a wonderful view from the top" - using 3-dimensional SEM

Bernard Breton
Cambridge University

The Private Life of a Biofilm

Pauline Barber
Eastman Dental Institute

A biofilm is an accumulation of bacteria and bacterial products on a surface; it may be beneficial, in medecine or immunology, or bad, in biocorrosion, biofouling or biodeterioration. It is affected by extremes of temperature, salinity, and pressure, and may be anaerobic.
(Edison Differential,.....microscope)
Stains used in the Unit:
Safranin O - Shepherd & Mitchell, J Ultr Res 1976, 54, 451-460
Ruthenium red - Springer & Roth 1973, J Gen Micro
Alcian blue - Shea 1971 J Cell Biol U51U, 611
Lysine in fixative - Jacques 1971 ?

There is always an organic cuticle between the plaque and the underlying tooth surface. The constituent organisms of the plaque can exclude other pathogens. An Actinobacter is the main pathogenic factor; it has bone-resorbing properties.

EM in Kenya

Clive Wells

His work is mainly concerned with Trypanosomiasis; which causes sleeping sickness in humans, wasting and lethargy in cattle. Indigenous species of animals are tolerant of it, not immune; domestic livestock die from it. The Africans will not kill sick cattle even if they are sterile, because wealth is measured by number of cattle. Horses etc. do not survive. The disease can be carried by lizards etc.

Chemotherapy may be used, but there have been no new drugs since 1950. There is one drug which cures and can be given prophylactically to produce relative immunity in large doses; but resistance is beginning to develop. The drugs are cytotoxic anyway.
Clive is working on the drug Samurin, which produces autofluorescence within the trypanosome. The fluorescence shows that it takes 18 hours for the drug to penetrate into resistant organisms, but only 15-30 minutes into a sensitive organism; it is then transported to the kinetoplast.
For EM autoradiography, fixative in a tube is covered by a layer of silicone oil, then the suspension of Trypanosomes in Samurin is layered onto this, and the tube centrifuged. A strong signal is obtained after only 15 seconds incubation.

EM visualisation of mycorrhizal fungal slime

Hilary Denny
Open University

She is working on heavy metal tolerance. Certain fungi are associated with the short roots of trees, with the mycorrhiza penetrating into the surrounding soil; these are Ectomycorrhiza. The trees with associated fungi are more tolerant of zinc; there is a lower concentration of zinc in the roots, but more in the roots. Within the roots, the fungi are intercellular.

Fixation etc are difficult because of the dense fungal mantle; freeze-substitution is a good method. She uses aluminium grids. The outer hyphae have little zinc, the inter-cellular hyphae have a lot. This suggests that the metal binds to the extra-cellular slime.
Ericaceous plants, including Bilberry and Rhododendron ponticum, have very fine roots, and a high tolerance to zinc in plants with associated mycorrhizae.

It is difficult to fix and demonstrate the slime because it is thin and easily lost - although often quite thick in places. Ruthenium red will demonstrate it. It forms links between adjacent hyphae. Dehydrate to 85% alcohol, then to LR White, with minimum agitation during processing.
A thick loose slime layer is more efficient at mopping up the metal.
She is working at the limit of sensitivity of EDAX, and hopes to try EELS.

Meteorites & micrometeorites down the microscope

Monica Grady
Natural History Museum

Meteorites are cold inside as they land. The iron cools more slowly than if coming from a blast furnace, so nickel can move within the lattice and can suck in trace elements.
In stony meteorites, some particles may have melted and quenched.
Back-scattered images show differences in chemistry.
Terrestrial processes will also cause alterations in the meteorite after it has landed; e.g. iron will oxidise.
If the silicates are dissolved out in hydrofluoric acid, diamond crystallites 3 mm diam will remain.
Every star, including the sun, has a stellar wind which blows particles away.
These diamonds are older than our sun; they do NOT come from our sun, as shown by the trace elements present.
A little green meteorite from Mars is composed of carbonates which were laid on a base in water; this can be shown because of the temperature and carbon dioxide content.
50,000 tons of extra-terrestrial material land on the earth every year.
Everyone is hit by cosmic dust once a day; these are usually fluffy particles, but larger melted ones can be found in Antarctica. These larger ones, "cosmic footballs" have glassy centres. The cosmic dust also samples the comets.
They may make holes in the outer layers of satellites - as also does space debris - as well as micro-meteorites.

Macrophage apoptosis in vitro & in human atherosclerosis

Laszlo Hegyi
Cambridge University

Atherosclerosis may begin in childhood; this can be shown by the condensation of heterochromatin. There is an unusual DNA polymerase; terminal deoxy-nucletidyl transferase.
Proteinase K incubation is essential on paraffin sections. For EM, Lowicryl HM 20 is used after freeze-substitution.

(funded by Cambridge Overseas Trust & British Heart Foundation ?)

TEM section-style images from an SEM

Geoff Richards
ASIF Research Institue, Davos, Switzerland

Uses back-scattered electron in Field-Emission SEM, Hitachi H 100 FESEM.
The block is trimmed and coated. The position of the back-scatter detector is important, bacuase we don't want topographical information; for this reason, also he uses 8 - 12 kV.
It is not necessary to use light microscopy first for topographical info. It is possible to invert the polarity black-to-white, to compare with the TEM image.
Microvilli can be seen sideways on. He also showed nails corroding in a femur. It is possible to section the specimen with the kV, but not yet possible to remove the information form areas above the "section".

One-day meeting 1995
List of Registrants

Paul Ansell	Hitachi Scientific Instruments, Finchampsted, Berks.
Iolo apGwynn	Biological Sciences, Univ. of Wales, Aberwystwyth
Emile Asselebergs	Philips Electron Optics, Cambridge
Pauline Barber	EMU, Eastman Dental Institute
Sue Barnes	EMU, Natural History Museum
Richard Blackburn	School of Environmental Science, Univ. of Greenwich
John Bredl	EMU, Royal Veterinary College
Tony Brain	EMU, King's College, Campden Hill Road W8 7AH
Bernard Breton	Engineering Dept.,Cambridge University
Paul Bunting	Oxford Instruments, Eynsham, Oxford
Steve Cham	Leica, Cambridge
Bill Clark	Agar Scientific Ltd., Stansted Essex
Don Claugher	Kingston-on-Thames, Surrey
Karen Chance	Inst. of Biol Sciences, Univ of Wales, Aberwystwyth
Graham Cliff	Manchester Materials Science Centre, UMIST
Che Connor	Inst. of Biol Sciences, Univ of Wales, Aberwystwyth
Terry Cooper	Taab Laboratories Equipment Ltd.,Aldermaston, Berks
Heather Davies	Biol Dept, Open University, Milton Keynes
Hilary Denny	Open University, Milton Keynes
Trish Dopping-Hepenstal	Dept Cell Pathol., St Thomas' Hospital
Barry Dowsett	CAMR, Porton Down, Wiltshire
Anne Drewe	Dept Microbiol. Charing Cross & Westminster Med Sch
David Gittins	PGT, Peterborough, Cambs.
Fathi Gowali	Histopathology, Charing Cross & Westminster Hospital
Terry Gradidge	Dept Neuropathology, Frenchay Hospital, Bristol
Monica Grady	Dept Mineralogy, Natural History Museum
Tony Gratian	Dept Cell Payhol., St Thomas's Hospital
Alan Gray	Dental Inst., London Hospital
Karen Gresty	Dept Biol Sci, Univ of Plymouth Drake Circus P
Nicholas Hathwag	Inst. of Biol Sciences, Univ of Wales, Aberwystwyth
Pippa Hawes	Inst. of Biol. Sciences, Univ of Wales, Aberwyswyth
Laszlo Hegyi	Dept Pathology, University of Cambridge
Tanya Hopcroft	Histology, BVS, Royal Veterinary College
Fiona Holt	Wye College, Ashford, Kent
Jo Hunt	Dept of Materials, Open University, Milton Keynes
Kevin Jennings	Smithkline Beecham Pharmaceuticals, Welwyn, Herts. The Frythe, AL6 9AR
Lynne Joyce	Agar Scientific Ltd., Stansted, Essex
Louisa Jones	EMU, Natural History Museum
Chris Jones	EMU, Natural History Museum
Mike Kelly	Dental Institute, London Hospital
Jill Lewis	EMU, Eastmand Dental Institute
Doug Lock	PGT, Peterborough
Patricia Lovell	Institute of Zoology, London NW1
Gareth Rhys Lloyd	School of Pharmacy, London WC1
Allister McBride	Leo Electron Microscopy Ltd., Cambridge
David McCarthy	EMU, School of Pharmacy
Graham McPhail	Histopathology Dept., St Bartholomew's Hospital
Hilary McPhail	EMU, Physiol. Dept., St. Mary's Hospital Med School
Roger Meadows	Inst. of Biol Sciences, Univ of Wales, Aberwystwyth
Nicky Mordan	EMU, Eastman Dental Institute
Helen Mrowiec	Inst. of Biol Sciences, Univ of Wales, Aberwystwyth
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Shelagh Reardon	Wye College, Ashford, Kent
Geoff Richards	ASIF Research Inst. Davos, Switzerland

Padmini Sarathchandra	Institute of Orthopaedics, Stanmore
Leigh Scott	Leica , Milton Keynes
Kevin Scudder	Gatan Ltd., Corby, Northants
Andrew Searle	Oxford Instruments, Eynsham, Oxon
Ian Shore	Histopathology, Charing Cross & Westminster Hospital
Arthur Smith	Bemax (UK) Ltd., Milton Keynes
Paul Southey	Imaging Associates Ltd., Thame, Oxon
Leslie Stump	Imaging Associates Ltd., Thame, Oxon
Sabrina Sukardi	BVS, Royal Vet College
Chris Walker	Philips Electron Optics, Cambridge
Clive Wells	ILRI Kenya PO Box 30709
Robert Whitenstal	Materials Dept. Queen Mary & Westfield College
Christopher Wilson	Inst. of Biol Sciences, Univ of Wales, Aberystwyth
John Yardley	Inst. of Biol Sciences, Univ of Wales, Aberystwyth
Hilda Zavaleta-Mancera	Inst. of Biol.Sciences, Univ of Wales, Aberystwyth

71 Total
17 Trade
54 non-Trade