SOCIETY OF
ELECTRON MICROSCOPE
TECHNOLOGY

TEA & TUITION

a problem solving session

on Wednesday December 10th at 2 pm 1997

at

The School of Pharmacy

(Brunswick Square)

Come and discuss your E.M. problems round the tea table with the experts.
These will be :-

Jeremy Skepper - manager of the Multi-Imaging Centre at the University of Cambridge has exceptional experience in all problems relating to specimen preparation, from fixation to the choice of resin for particular applications, and the variety of techniques required for successful cryosectioning. He also has expertise in the field of stereology.

Carole Winters - electron microscopist in the School of Pure and Applied Biology at the University of Cardiff specialiizes in quantitative XRMA using cryo techniques on biological thin specimens; this includes quantitative and qualitative mapping and point analysis. She is also involved in qualitative and low vacuum techniques in the SEM.

Peter Jackson - Head of Research Histology at Leeds General Infirmary was involved in the development of silver enhancement techniques for immuno-gold labelling. He has particular experience of archival human materials and employs microwave stimulation of immunocytochemical reactions.

Brian Wells - of the John Innes Institute, Norwich has over 30 years experience of low temperature embedding of plant tissues. He will demonstrate a tissue handling device which facilitates solvent exchange during specimen preparation for immunocytochemistry. He has recently developed high pressure freezing and freeze-substitution techniques which have been successfully applied to *Arabidopsis.*

There will a charge of £2 per head for tea, savouries and cakes.

At 4.30 p.m., mince pies and sherry will precede the Annual General Meeting

RSVP to Dr Jill Lewis, 19, Bellfield Avenue, Harrow Weald HA3 6ST
(0181 428 4264) by Friday, Dec 5th please.

Tea & Tuition 10 December 1997 .S.E.M.T.

Brian Wells
John Innes Institute, Norwich

Alcohols precipitate proteins.
Below -4°C, the volume of ice changes dramatically. Ice crystals are less of a problem in plants than might be expected, because the cell sap is not pure water.
Plant tissue may be cooled very slowly, to produce very small crystals; or very fast, 1000°K in 0.1 seconds.

He recommends the Hauser slammer, which has an electro-magnetic coil to hold the tissue in contact with the liquid-nitrogen-cooled surface. (the Polaron model allows the tissue to bounce off the surface)

Spray-freezing of algae can be done with an artists' air-brush, into liquid propane in an Eppendorf tube.

For jet-freezing, place the specimen between 2 copper discs, and spray the cryogen from both sides simultaneously; OK for suspensions.

For Arabadopsis, a weed with small chromosome number, he uses the Balzers high-pressure freezing machine, cost £40,000. Freezing with hexadecane or n-heptane.

The liquid nitrogen container made by Taylor Walker, available from Agar Scientific, is allowed on plane and car.

The Eppendorf tube can have 25µ nylon mesh heat-sealed across it on a hot plate.

LR White can be polymerised using the catalyst of K4M.

A slow Progressive Lowering of Temperature schedule is used for plants; a faster schedule can be used for animals.

A nylon mesh, Plastok, and vinyl tube are available from Plastok, 79 Market street, Birkenhead, Wirral LE41 6AN

Temperature probes can be obtained from Radiospares.

Cable marker, cat.no. 554-636 etc, from RS Components Ltd. Box 99, Corby, Northants NN17 9RS

Alice Warley

99% of XRMA is done by materials scientists for SEM applications.
Quantitative results can be obtained using a metal grid.
She locates sodium, potassium, calcium, magnesium on heart tissue.

The EM detector + computer (? +EM) cost £30 - 40,000

She started with viruses infecting cells; they do not alter the membranes.

Sodium concentrations near a boundary controld procedures across membranes.

She wants STEM to do mapping with.
She can deal with specimens up to ½u thick, but then loses spatial resolution

Jeremy Skepper
Multi-Imaging Centre, Cambridge

To inactivate viruses for negative staining, 0.1% glutaraldehyde could be added to the resuspended pellet. If the pellet is suspended in phosphate buffer, calcium could be precipitated; but faeces would probably be OK. If there are problems, try resuspending in Hepes or Pipes.

Lowicryls:
the K series are slightly less hydrophobic than the H series. Soem antibodies work with one, others with the other, or perhaps with both. HM20 is easier to handle and to cut, and can go down to -50°C; K4M cannot be used at so low a temperature.
Try the antisera at the light microscope level first. Find the conditions fro fixation; then with HM20, the concentration at which it reacts strongly.

Over a period of time, formol saline becomes contaminated with methanol and formic acid.
Formaldehyde for fixation should be freshly prepared from paraformaldehyde; this gradually also becomes contaminated.

"Fine Structure Immuno-cytochemistry" - Gareth Griffiths; pub. Springer.
(He is biased against horse-radish peroxidese)
Glauert is revising the Fixation book with Peter Lewis.

Cryostat unfixed sections - it is essential to have a good reaction with a new antibody before seeking thde best fixation method; go through the series: unfixed / acetone / formaldehyde / cryostat → paraffin.
Plastic sections label only at the surface, so you need x 100 concentration for light microscopy.
Polyclonal antibodies are liable to produce much more gubbins because of the many epitopes.

You may have to apply antibody for ½ hour, wash, and re-apply antibody, several times, rather than apply once for many hours, in order to get rid of low avidity.

The freeze-substitution apparatus from Leica is much better than the Balzers one, and much better than the RMC one.

There is no rule of thumb in immuno work; each antibody reacts differently.

Horse-radish peroxidase can be used as a tracer of damage etc, but it is diffusible.

Nanoplast, a melamine resin, can use dehydration through the resin monomer; it is excellent for high-power work; it is not water-soluble.

HM20 and K4M sections must be put on a support film.. Irradiate the whole grid first at low intensity, then go to a higher magnification.

Peter Jackson
Research Histology, Leeds General Infirmary

Neil Hand, of Nottingham, is developing his own resin. At a Path Soc meeting in 1989 he claimed to be able to use 100 more antibodies with this than with any other. It has a methyl methacrylate base.

Glycol methacrylates, when polymerised, cannot be removed, and will continue to polymersie. Methyl methacrylates are soluble in xylene. 1½ - 2 um sections can be used for light microscopy, for morphology and immuno-cyto-chemistry, with formaldehyde fixation and alcohol dehydration.
Methyl methacrylate + dibutyl phthalate + amine catalyst will polymerise with no heat involved.
On biopsies, a colleague in Scotland uses less dibutyl phthalate, to get EM sections. This does not move much under the beam. It is NOT necessary to wash out "stabilizers".

Direct phone line 0113 392 64 68 for details of methacrylates.

For antigen retrieval on archival material, use;
pressure-cooking for nuclear antigens
microwaving for cytoplasmic antigens
This is better than proteolytic techniques, which are more complicated. Also these are now routine techniques. Use citrate buffer pH 6, or 0.1 M EDTA pH 6.

He recommends Superfrost + slides from BDH; or Apes-coated slides from Sigma - dip the slide in acetone and let it dry.

Optimum fixation time in formaldehyde is up to 10 days for biopsies; if you fix for only 24 hours, fixation is incomplete and the subsequent alcohols will also play a (less efficient) part in the fixation process.