21-10-94

SOCIETY OF
ELECTRON MICROSCOPE
TECHNOLOGY

BACK TO BASICS

FRIDAY OCTOBER 21st 1994

INSTITUTE OF DENTAL SURGERY
GRAYS INN ROAD

2.00 p.m.

Programme

2.00 "Fact or artifact - interpretation of images"

Dr Iolo ap Gwynn (University of Wales)

2.40 "Principles of fixation"

Dr Jeremy Skepper (University of Cambridge)

3.10 TEA

3.30 "Historical aspects of Electron microscopy"

Alan Agar (Helmsley, York)

4.10 " Safe working for electron microscopists"

Dr Garry Burdett (Health & Safety Executive, Sheffield)

4.50 Final discussion.

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I hope to be present at the meeting on 21st October -

Name .....

Address .....

.....

Please send to the Secretary -

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BACK TO BASICS
Abstracts

" Fact or artifact " - interpretation of images

Dr Iolo ap Gwynn (Univ of Wales)

With so many institutions now boasting the availability of EM facilities we are tempted to assume that all who use them are fully conversant with the process of interpreting the images they have obtained. Sadly, this is far from the truth. All too often one is confronted with manuscripts where EM images are proudly presented is some apparent attempt to legitamize the work by decorating it with the product of such a high-tec approach, while at the same time giving little or no attention to the interpretation of such images.

It is impossible to interpret such information on the basis of previous, unrelated experience. In both SEM and TEM, consideration must be given not only to the ways in which specimen preparation, in terms of preservation and contrast generation, affects the image but also the way in which electrons interact with the specimen. None of the images we have to deal with, in any real sense, represent the original living biological object of attention. They are all artifacts of one sort or another. The skill of meaningful interpretation lies in our ability to interpret these artifacts. When all this has been taken into account, only then can the problem of assembling the obtained image information into a description which makes biological sense in at least four dimensions be addressed. The electron microscopist often needs to operate as an interpretative interface between the data produced and the naive researcher, however experienced and knowlegable that person is in other fields. This is more of a problem in electron microscopy than in many other disciplines because most of the data is in the form of images, and everyone thinks they can understand "pictures".

" Principles of fixation "

Jeremy Skepper (Dept of Anatomy, Univ of Cambridge)

The Oxford English Dictionary defines fixation as : The action of depriving of volatility or fluidity, or The process of rendering solid, a liquid or semi-liquid substance. To examine biological systems by electron microscopy the speed of that process is of paramount importance. It is also necessary to know to what degree the molecular structure of components of that system are changed by fixation. This has profound implications upon the microscopist's ability to glean information about the mechanical and chemical structure of a biological system.

Electron microscopical studies can be loosely split into four categories: ultrastructural, cytochemical, enzyme histochemical and immunocytochemical. Each have the initial requirement for some type of fixation, be it chemical or cryo-immobilization. I shall concentrate on chemical fixation and discuss the central requirements of any fixative. I shall then describe the methods we have chosen to accomodate the four categories of investigation named above and their rationale. There is much room for variation of fixation parameters and I hope to identify logical ways of reaching the appropriate compromise.

"Historical aspects of electron microscopes"

Alan Agar (Sproxton, Yorkshire)

A comparison is made between the designs of the earliest produced microscopes and related to the differing technologies available and the designers own priorities. Some of the recent important developments are traced to their origins.

" Laboratory safety "

Gary Burdett (Health & Safety Lab., Sheffield)

This sermon on the "Back to Basics" theme will first look at the "new" philosophy behind Health and Safety that has been handed down to us via European Directives on tablets of stone. We will then see how it has been translated into annual festivals such as safety audits and other more regular rules of observance via the "New English Edition" of Approved Codes of Practice for the Management of Health and Safety. The unrighteous amongst us will then be instructed in the true meaning of the observances and how we may better follow them. Already COSSHed and intoxicated by the Six Pack we will investigate how we as individuals can extend our stay in this temporal realm.

Fact or Artefact - Interpretation of Images

Iolo ap Gwynn

Interpretation has to be for the benefit of other people too. Memory works by association. Microscopy images cannot be interpreted on the basis of any other previous experience.

We must take into account the preparation tech niques; magnification; (most journals now insist on a scale bar on micrographs - much better than giving magnification as X...; otherwise one can use the unit cell membrane as a 10 nm scale. Bear in mind resolving power, as against resolution (1/10 of section thickness). Contrast can be determined by the stains and labels; or negative stain; or unstained. We are now using acrylics again, so are getting the old problems - compression etc.

Don't be in too much of a hurry to interpret the images which you have recorded. Don't depend on a single technique; in the past this has led to disputes about the reality of microfilaments, microtubules; mesosomes and myelin bodies are know considered to eb artefacts. The small depth of field may give apparent gaps in membranes because of the angle of sectioning. Immunocytochemistry is of great interest to the molecular biologists; it requires numerous controls.

With acrolein fixation, the membranes stand out well.
Interpretation of SEM images is even more difficult. The secondary electron image will contain back-scatter from areas deep within the specimen.

Principles of Fixation

Jeremy Skepper

What do we want to fix, and why? We amy be fixing for ultrastructure; cytochmistry; enzyme histochemistry; immunocytochemistry.
The fixative contains buffer, one or more cross-linking agents, and additives. The optimal concentration of glutaraldehyde is determined by the maount of free amine in teh tissue; oxygen must be available. If there is too much glutaraldehyde, the aldol reaction which actually be slower. Acrolein penetrates and acts faster.

Use dextran or PVP to increase osmolarity & viscosity.
Phosphate buffer is cheap, but not much else. PIPES and HEPES buffers cover a good range of pKa; charges basic sp (?) which do not pass unfixed membranes. They do not bind or precipitate monovalent or divalent cations, so magnesium or calsium can be added to the fixative. Expensive! Imidazole, which has

uncharged basic species, may be a useful alternative, but ti reacts with glutaraldehyde and osmium, so it must be mixed immediately before use. It enhances lipid retention.
After osmium fixation, wash in potassium ferro- or ferri-cyanide in PIPES for up to 2 hours; then in buffer alone.

Ether produces stressed respiratory structures; it is better to use a calming anaesthetic. When perfusing, have a short line from the fixative jar. If using an immersion fixation, be prepared to modify the technique.

Historical Aspects of Electron Microscopy
Alan Agar

Ruska & Von Borries did applications work from the beginning - thus rousing interest in their ideas. Early specimen drives were by means of a spring and two rods at 120'; now an orthogonal push is sued.
Metro-Vickers - talk/booklet 'Where to kick your EM'
Brass is not non-magnetic.
In the late 1940s, everyone was very open about developments, perhaps a reason why development was so fast.
The early Phillips and RCA microscopes did not have a specimen air-lock, to simplify the vacuum system!
Holography for high resolution was proposed by Gabor in 1947. Then in 1986 the idea surfaced again at Tubingen. 1949 Castaing rpoposed XRMA.

It pays to read the literature, from the early days on. Don't be over-awed by The Boss!

Safe Working for Electron Microscopists
Garry Burdett

From 1974, there was concern about safe systems, leading to COSHH. There is a chemical handbook of EC Risk & Safety Phrases.
List the: Substance, Form, Process, Hazard, Location, Storage, Amount.....
Classification, Packaging & Labelling Regulations 1984
Chemical (Hazard Information & Packaging) Regulations 1993 - applies to the suppliers; we hope it also gives us information.
Hazard is the potential to be toxic; Risk is the probability of the happening.
(Germany doesn't discriminate between hazard & risk; the UK and Europe do)
CHIP 1993 implements 9 European dire ctives.

COSHH is a hazard assessment; & now is a risk assessment too.

Work activity/ Hazards/consequences/Risk/Controls/Risk with controls/Activities to maintain controls

Hazard: 3 - major - death or major injury

2 - moderate - off work for more than 3 days

1 - slight - off work less than 3 days

Risk is graded similarly.

When you do an assessment, WRITE IT DOWN !!

CHIP 2 is on the ways; COSHH 1994 also - will cover biological hazards - a directive to COSHH biological systems.

Legislation on the disposal of materials comes under CHIP

There will soon be regulations about fume cupboards.