Society of Electron Microscope Technology

(Http://www.semt.org.uk)

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A ½ Day Meeting:

MULTI-DISCIPLINARY APPROACH

Wednesday 13th December 2000
School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX

2.00 p.m. Introduction – The Chairman

2.05 pm Which Fluorescent Microscope and when ?
Alan Entwistle (The Ludwig Institute for Cancer Research, London)

2.35 pm Synaps Counting : How exciting can it be ?
Claire Kendal (The Open University, Milton Keynes)

3.05 pm Tea / Coffee.

3.30 pm The use of Microscopy as an analytical tool at RSSL.
Jill Webb (Reading Scientific Services Limited)

4.00 pm Investigating Phylogeny – The use of microscopes in Nannoplankton research.
Markus Geisen (Palaeontology dept., Natural History Museum)

4.30 pm Sherry and Mince Pies, followed by the

ANNUAL GENERAL MEETING

For further information and registration, please contact the Secretary (details above).

Which Fluorescent Microscope and when ?

Alan Entwistle
Ludwig Institute for Cancer Research, 91 Ridinghouse Street, London

Fluorescent microscopes can currently be divided broadly into: wide-angle or conventional; confocal; demi-focal; Tandem Scanning confocal; Line Scan confocal; Moving Mask confocal.
It is not always necessary to mess about with the image in a computer. The speed of needed acquisition of image is often the determining factor in choice of microscope. For a given image, a wide-field microscope may capture the image in 15 seconds, whereas the confocal would need 3 minutes.

The choice is for resolution versus speed. On confocal microscopes the resolution is always poor anyway. For a good signal/noise ratio, you need a short time (??)

Confocal microscope is very useful for 3-D anatomy of small anatomical structures, eg alveolar duct of lung; and also for very large specimens, because it eliminates stray light.

2-photon fluorescence is even slower, but can penetrate deeper into the specimen, because it uses infra-red which has a better penetration.

It is not clear whether a living specimen remains truly viable after multi-photon fluorescence microscopy. Photo-bleaching occurs more rapidly.

De-convolution in the computer can be misleading. There is no reason why 3-D co-localisation should not be done with the wide-field microscope.

Confocal for: very highest resolution
intra-cellular structures in thin sections
relative proximity of several different molecules

wide-field for: speed
greater sensitivity

2-photon excitation for deep probes

Demi-confocal has advantages intermediate between wide-field and confocal.

If masks are used, the resolution of the confocal is obtained, but the speed of acquisition of image is increased.

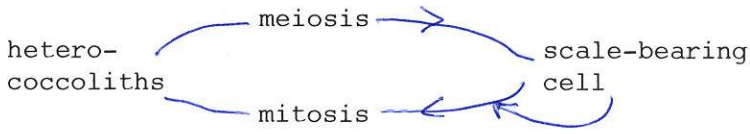
If an auto-fluorescent subject is being examined, eg teeth, wide-field techniques do not get rid of the auto-fluorescence.

Investigating Phylogeny - the use of microscopes in Nannoplankton research

Markus Geisen
Palaeontology Dept., Natural History Museum

These organisms are micron scale - about 20 microns diameter. They include Coccolithophorid, of calcium carbonate, from the Triassic (various species); there is a good fossil record. Photosynthetic, & marine - a major contributor to the marine primary production, and important in the carbon cycle. They can be cultured, but this is difficult.

In these investigations, molecular phylogeny versus fossil record. Cigarette buts are a good narcotic. These organisms contain a lot of CO_2 plates, which are produced inside the cell.



There may be cryptic speciation, and intra-specific variation; this is not yet sure.
Plate are produced at a steady rate, and are morphologically constant for a single species.

The use of Microscopy as an analytical tool at RSSL
Jill Webb
Reading Scientific Services Ltd.
Lord Zuckerman Research Centre, Whiteknights, Reading RG6 6LA, POB 234.

The RSSL has many depts; including:
Emergency Response Service
Microscopy & Foreign Body Analysis
Spectroscopy - FTIR, GC, NMR, Mass Spectroscopy, etc, etc.
Pharmacy, Food Industry Training etc
SEM (+ FTIR) - Jeol + Hexland

Few metals are found in food resulting from manufacturing processes, because the metal detectors are so good.
Glass - can often be swallowed and voided without problem. Pyrex rims are found quite often, and are borosilicate. Deposits on the glass may indicate their origin; under crossed polars, a bimodal population is typical of wheat starch; peanuts are bi-refringent both unstained and with Toluidine Blue stain.

FTIR will distinguish $CaCO_3$ and Ca oxalate in the residue from drinks. But the FTIR of Ca phosphate and starch have a similar pattern, therefore iodine stain is used for starch, and also XRMA. Similarly protein and nylon give a similar pattern.

Magnsium Ammonium Phosphate hexa-hydrate (= struvite) grows spontaneously in canned salmon and looks like glass.

It is necessary to have the specimen still at the end of the analysis, as it may need to be presented in court.
Polyethylene - by FTIR the same result is given for solid and for sheet (bag). A thermogram Differential Scanning Calorimeter is needed to distinguish high or low density polyethylene.

In pharmaceutical products, steel and PTFE may sometimes be found. In a protein-containing pharmaceutical liquid, a floater of silicon became coated with protein.

She seldom buys from the bargain baskets in shops now, as she knows all the tricks to banjax things - holes in tins under the label etc.

Synapse Counting: how exciting can it be ?
Claire Kendal
Open University, Milton Keynes

She is studying neurodegenerative conditions, including Alzheimer's disease. This can be early onset, before the 5th decade, or late onset. It shows as an impairment of short-term memory, spatial disorientation, aggressive &/or depressive behaviour.

Finally all memory systems fail; there is aphasia, apraxia, agnosia, and all intellectual functions fail, and the patient is increasingly incapacitated. With early-onset Alzheimers, there is a direct genetic cause in less than 7% of cases. There is a dose-effect risk of 40-50% for early and late onset with the gene for Apolipoprotein β.
A sporadic 50% of cases are genetic/environmental - age, head trauma, hypothyroid, cerebrovascular disease, Alzheimer's.
Protective factors are: Vitamin E (as free radical scavenger), mental exercise, Hormone Replacement Therapy.

The classic lesions are: regional atrophy, weight reduced to less than 30-40% of normal, widening of gyrate and sulcate rifts, enlargement of the ventricles. Plaques and tangles are found, with amyloid in the centre. There is neurone and synapse loss, beginning in the temporal lobe, hippocampus and frontal cortex.

The amyloid precursor protein is the parent molecule of β-amyloid (Aβ); the gene is found on chromosome 21. Amyloid Precursor Protein is the cell surface receptor, for cell adhesion, synaptic nodules, memory, & nervous system function. Aβ is found in the centre of the plaques, and in the deposits in the brain and vasculature in Alzheimer's.

There are transgenic models - mice which over-express the genes coding for APP; these show an increase in production of AB, leading to some lesions of Alzheimer type and comparable behavioural changes. It is possible to use these to test therapeutic drugs.

The mouse project Tas 10 over-expresses APP. It produces plaques, Aβ deposits, neuronal loss, synapse loss, and Atrophy. This is being studied by EM, LM, confocal, & stereological techniques.
There is atrophy of the hippocampus; loss of neurones in CAI and dentate gyrus; synapse loss in the middle molecular layer, increase in the mean apposition zone - fewer synapses but larger. The Cavalieri method is used for the ratio of synapse number/volume, for brain and hippocampus. There is also a method for calculating the Apposition Zone measurements.

Comparing Ageing and Alzheimer's - there is no synapse decrease; the Apposition Zone size increases (in ageing ?)

In these mice, there is some degeneration at 6 months, a lot by 18-24 months. Synapse density, Apposition Zone Area, neuronal number, hippocampus size, and Aβ plaques are being studied. There is more variation in the morphology with younger mice; less variation within the groups as the mice age.

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