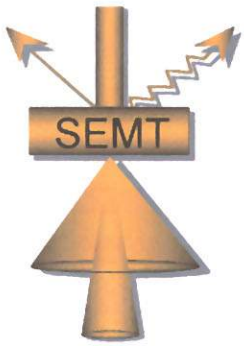

Society of Electron Microscope Technology

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

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Half - DAY MEETING

Wednesday 6th November 2002

at

The School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX

12.30 - 1.55	**Registration**, including a ***Buffet Lunch***
1.55 - 2.00	**Introduction** : Chair, Barry Dowsett.
2.00 - 2.30	**Electron Microscopy of Human Hair : Ethnic differences and disease states.** *Prof. David Ferguson. Path. Dept. John Radcliffe Hosp.Oxford*
2.30 - 3.00	**Diagnostic Electron Microscopy in Primary Ciliary Dyskinesia.** *Ann Dewar. EM Unit, Royal Brompton Hosp. London.*
3.00 - 3.30	**Tea & Coffee**
3.30 - 4.00	**In situ hybridisation at the Ultrastructural level : localization of bacterial rRNA in Algal cells using biotinylated probes.** *Gabrielle Kennaway. Biological Sciences, Univ. Westminster.*
4.00 - 4.30	**Variable pressure SEM for the examination of pulsed-laser cleaned natural history samples** *Chris Jones. Natural History museum, London.*
4.30 - 5.00	**Annual General Meeting**.
5.00 - 6.30	**Wine Reception.**
6.30.	**Conference Dinner.**

S E M T Half-Day Meeting 6 November 2002

EM of Human Hair: ethnic differences & disease status
Prof. David Ferguson
Dept. Pathology, JohN Radcliffe Hospital, Oxford

There is still a role for "bog-standard" EM in diagnosis.
Toxoplasma gondii survives 2% H_2SO_4. The acute phase (in internal host) proliferates in many cells including macrophages; it is destroyed by the immune repsonse; sensitive to drugs. Chronic infection leads to cysts in the brai & muscles; here it is viable for the life-time of the host; & is resistant to drugs; a source of infection in the immuno-compromised. 30% of Britons have the infection; it is lethal in AIDS patients. There are stage-specific antigens. We must compare like with like - intra- or extra-cellular. Some things e.g. enolase may have more than one function, - perhaps in gene regulation as well as glycolytic function.

The medulla is of variable size; granules in cortex; cuticle of overlapping cells. The granules are micro-fibrils. African hair has oval cross-section, & forms coils of 1½ mm diam. Causcasian hair is brushed a lot, & usually shows much "weathering" at the ends; Asian hair does not.
"Uncombable hair" syndrome - triangular hairs; these are less easy to bend than circular cross-section.
For TEM, the hairs are dehydrated through alcohols to resin; the problem is keeping the hair in the section; use filmed grids.
Hair grey or black - depends on the number of melanosomes.

Diagnostic EM in Primary Ciliary Dyskinesis
Ann Dewar
EM Unit, Royal Brompton Hospital

Sperm cilia have dynein arm; if there is situs inversus in one of the pair of arms, the sperm is immotile. Similar problems can be found in respiratory tract cilia; also atopic pregnancy, etc; probably under-diagnosed.
Their bank of specimens includes ciliary brushings from 10 years ago.
Cilia ar4 usually found in tufts; sections can miss these eassily.
There are many possible abnormalities, some without visible abnormalities.
e.g. strength of beat of outer arm; form of beat of inner arm.
Commercial software is available for the Markham rotation technique; Carson 2000; Escudier 2002.

In situ hybridisation at the ultrastructural level: localisation of bacterial rRNA in algal cells using biotinylated probes
Gabrielle Kennaway
Biological Sciences, University fo Westminster

An EU study on harmful toxic blooms, leading to toxins in shellfish from the diflagellates. The bacteria in the algae are symbionts, giving worse toxins. The dinoflagellate wall is of cellulose; it is difficult to get the probs into the cell, even using cellulase.

Fixation in 2% formalin + 0.05% glut makes a big difference to the perm-eability; + lysinne + periodate. The pellets are included in agarose.
Embed in Unicryl, because it tears just in front of the knife & leaves the protein exposed.

3 oligonucleotide probes were used, with post-embedding hybridisation. A constant temperature of 45'C is critical. No pre-hybridisation steps are necessary with Unicryl.

The nucleus has giant chromosomes. (With LRWhite, the nucleus always pulled out !); the cells are packed with bacteria, & also other dark stuff in the cells was labelled (compare the bacteroids with the cause of root nodules; here is an infection tube of bacteria in alga, so it is labelling). There is background because the chloroplasts have an evolutionary bacterial history. The label is on the bacteria, not on the chromosomes.

Hybridisation solution steps are simple but stringent, depending on the temperature & salinity, & number of sites.

Variable Pressure SEM for teh examination of pulsed-laser cleaned natural history samples

Chris Jones
Natural History Museum, London

VP SEM can also be used for assessing the condition of the specimens; removal of dirt, varnish etc; damage, eg fixing label & sealing over, plant treatment against pests; matric around specimen.

The traditional methods: brushing off dust just spreads it, & may be abrasive; steam pen for labels & varnishes; airbrasive (= micro-blasting) for matric round fossils - match the hardness, or you can damage the specimen; solvents (on specimens consolidated with chemicals or glued together); solvent gels (art conservators); all amy be OK for some applications.

Laser source - Neodymium Yttrium Aluminium Garnet; monochrome, visible green or infra-red. spot size less than 1 mm - 1 cm; Q-switch laser for pulse; minimum contrast - non-mechanical.
Showed bryozoan fossil, stained with ink, in limestone.

If a laser is focussed on dirt, the dirt will heat & expand rapidly, & the resuatnt force pushes the aprticle away.
showed: dung on dung beetle;
mercury compound on old herbarium specimen;
wasp's nest
butterfly
beetle - too much had been removed, because the specimen was dark-coloured.
dired leaf - grains of cinnabar removed OK;
wasps' nest - dirt removed OK to elave the structure intact.
butterfly - focus to 1 mm; large particles removed, but some small particles also removed.
It is possible to remove an Au coating from a bone speciemn moderately well.

We need to establish a method for quantification of laser absorption for infra-red & visible green wave-lengths by testing a range of coloured coatings & substrates; also optimum working distance (dependent on wavelength).