



ACEM

MicroOZcopy Down Under

John Stirling is ACEM's most distant member as he works in Anatomical Pathology at Flinders Medical Centre, Bedford Park, South Australia. John is heavily involved in the Royal College of Australasia's (RCPA) EM Quality Assurance Program and we are privileged to have his personal views on the state of electron microscopy in Australia.

EM down under - a personal view



John Stirling

Electron Microscope Unit, Flinders Medical Centre, Bedford Park, Adelaide, South Australia

Greetings from the land of Oz! As ACEM's only Australian member, I have been asked to give you a brief overview of electron microscopy in our currently very sunburnt land.

I came to Australia in the mid 1980s when diagnostic TEM was in full swing. At that time TEM was thought of as essential for the diagnosis of renal disease, a wide range of tumours and infectious diseases; negative staining was the gold standard for viral diagnosis. How things have changed! If only antibody probes hadn't been invented! Similar to the situation in the UK, the use of TEM in diagnostics has declined as affinity probes and molecular techniques have improved. However – TEM is certainly not dead yet. In Australia, TEM remains an essential diagnostic tool, particularly for renal biopsies, primary ciliary dyskinesia, muscle biopsies and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). We also continue to evaluate a small number of tumours (particularly reprocessed material from wax blocks) and to examine a small number of samples for infectious organisms. And, as might be expected, the range of applications utilised in any particular centre depends on the expertise and interests of the unit's scientific staff and pathologists. Scientists (assisted in some centres by senior technical staff) carry out the bulk of diagnostic examinations. Pathologists now rarely view specimens personally; for reporting purposes they rely on the images provided to them. Furthermore, laboratories are abandoning traditional film and hard copy images and converting to digital imaging. For example, in my own unit we scan our negatives and store them as 600 dpi jpeg files. The digital format facilitates easy internal access and we routinely email 300 dpi images to our interstate customers.

In respect to workforce review, unlike the UK situation there has been no national workforce review and no attempt at succession planning or rationalisation at the national level. However, in individual States diminishing specimen numbers, ageing staff and equipment and the high cost of maintaining a dedicated TEM laboratory have had the obvious outcome – unit closures and consolidation.

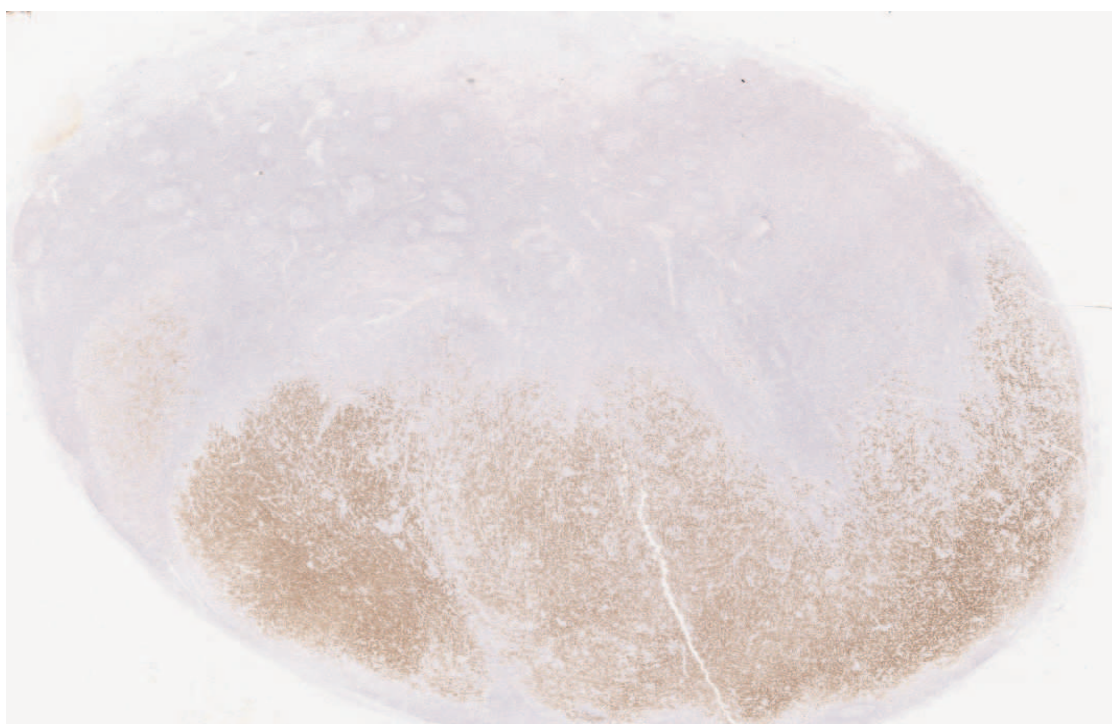
Furthermore, scientific and technical staff are increasingly required to multi-skill and cannot be expected to have the in-depth knowledge of the current 'dedicated' TEM workforce. In this climate professional quality assurance programs are essential for maintaining good laboratory practice and quality outcomes. I am pleased to see that QAP programs have been initiated by ACEM and the Royal College of Pathologists Australasia through their professional quality assurance group RCPA Quality Assurance Programs Pty Ltd. Also inherent in maintaining a viable diagnostic TEM service is fair payment for service. Currently, the payment we receive for a standard TEM specimen (~UKP80) barely covers staff costs, let alone equipment and infrastructure.

As for local groups that represent the interests of diagnostic electron microscopists and provide forums for meeting and exchanging news and views – these are now nonexistent. Our local EM society (the Australian Microscopy and Microanalysis Society Inc (ASEM)) now focuses mainly on microanalysis and materials science. It seems that ACEM is probably the sole voice of diagnostic TEM practitioners and I congratulate the founders – ACEM is an important forum for those interested in diagnostic TEM, an application that will continue to play an important and vital role in patient care for some time! Perhaps I'd better start recruiting more 'Down Under' members!

Value of dewaxing for EM in distinguishing carcinoma and cytokeratin-positive lymphoma.

Brian Eyden PhD, Department of Histopathology, Christie Hospital NHS Foundation Trust, Manchester

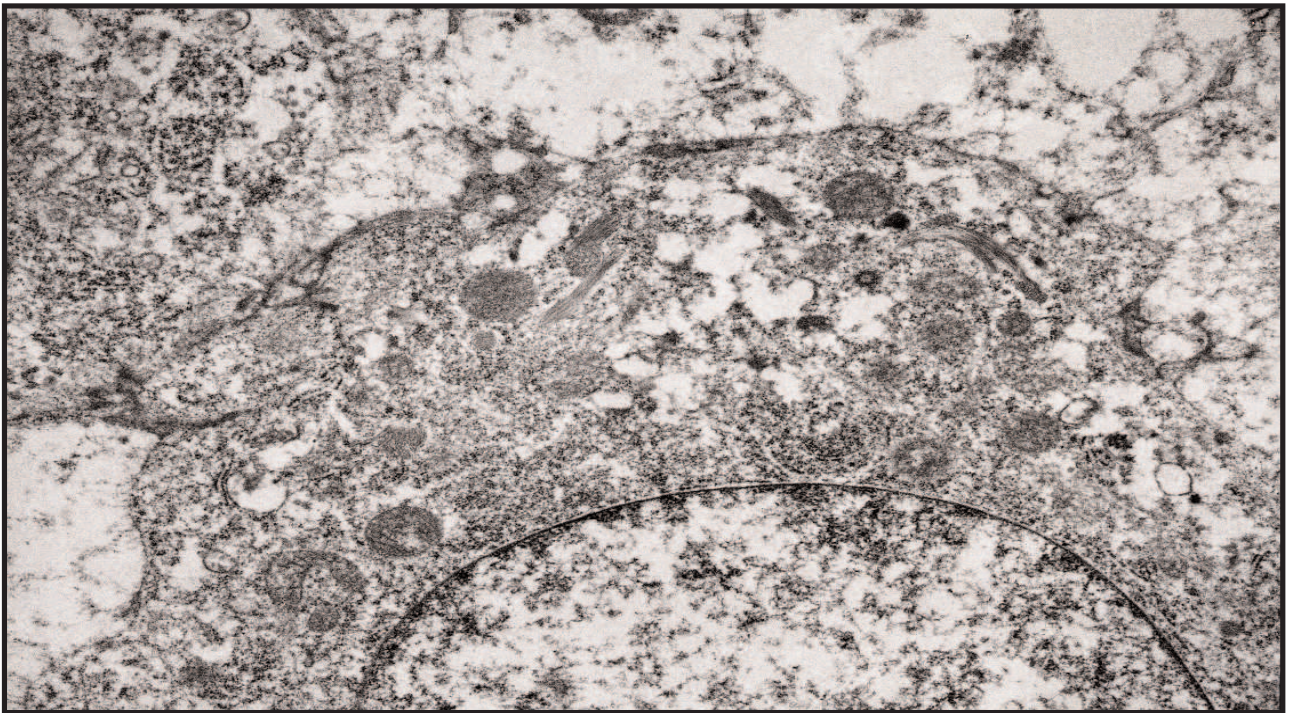
Lymphoma rarely needs electron microscopy for histopathological diagnosis, but an ultrastructural input can be useful in the distinction of a cytokeratin-positive lymphoma from carcinoma. We recently encountered a case of a lymph-node tumour with a large-cell morphology, and where the differential diagnosis was carcinoma versus lymphoma, the distinctive features of these two tumours being inapparent in H&E sections. Immunostaining was carried out and cytokeratin and BerEP4 (both convincing epithelial immunomarkers) were positive. Lymphoma immunomarkers (for example, leucocyte common antigen) were difficult to evaluate because of the intermingling of neoplastic with reactive lymphoid cells. Immunoglobulin gene re-arrangement studies were negative. Consequently, specialised ancillary techniques made little impact on the differential diagnosis, which was now carcinoma versus cytokeratin-positive lymphoma.



Electron microscopy of tissue retrieved from formalin at surgical cut-up gave large cells with plentiful rER, and no epithelial features, suggesting a plasmablastic phenotype and supporting one of the proposed light microscopy diagnoses, lymphoma with plasmablastic differentiation. However, one pathologist of several to see this case was unhappy at this interpretation given that the level of cytokeratin staining was perhaps too high for lymphoma, and because of the BerEP4, which is regarded as highly specific for epithelial differentiation.

It was therefore decided to perform further sampling for electron microscopy and in particular from an area of the wax block demonstrated to be cytokeratin positive. The first sampling of formalin fixed tissue had been taken from the residue of tissue at the surgical cut-up, and was unknown in terms of its cytokeratin immunoreactivity. We therefore cut another section from the wax block, stained it for cytokeratin, and then targeted a positively stained area for further electron microscopy sampling. This procedure led to the finding of tonofibrils and support for the diagnosis of carcinoma.

Usually, in diagnostic electron microscopy, retrieval from the wax block is avoided because of the well known damage to tissue ultrastructure in this process. However, here is an example where use of a selected area of the wax block, defined immunohistochemically, is to be preferred and offers a greater chance of identifying diagnostic organelles. In ultrastructural terms, the tumour was a poorly differentiated carcinoma, containing tonofibrils but lacking other epithelial features such as desmosomes, basal lamina and lumina, while the final histopathological report was *undifferentiated carcinoma from unknown primary site*.



Next Issue

Any contributions for the next newsletter should be sent to Alan Curry. E-Mail alan.curry@cmmc.nhs.uk or at dracurry@btinternet.com.

ACEM meeting at PathSoc Glasgow 2007



The Glasgow meeting had a truly international feel with speakers from several countries taking part.

There are more pictures of the Glasgow meeting available as a Powerpoint. If you'd like this Powerpoint then email **Bart.Wagner@sth.nhs.uk**. If any of you want any particular ones in an uncompressed format, just let Bart know which ones and he'll send them to you.

Publications

Curry A, Mudhar HS, Dewan S, Canning EU, Wagner BE. (2007). A case of bilateral microsporidial keratitis from Bangladesh - infection by an insect parasite from the genus *Nosema*. *J med Microbiol* 56: 1250-1252.

Sagoo MS, Mehta JS, Hau S, Irion LD, Curry A, Bonshek RE, Tuft SJ. (2007). *Microsporidium* stromal keratitis: in vivo confocal findings. *Cornea* 26: 870-873.

Canning EU, Curry A, Hill SLL, Okamura B. (2007). Ultrastructure of *Buddenbrockia allmani* n. sp. (Myxozoa, Malacosporea), a parasite of *Lophopus crystallinus* (Bryozoa, Phylactolaemata). *J Eukaryot Microbiol* 54: 247-262.

Findlow H, Vogel U, Mueller JE, Curry A, Njanpop-Lafourcade B-M, Claus H, Gray SJ, Yaro S, Traore Y, Sangare L, Nicolas P, Gessner BD, Borrow R. (2007). Three cases of invasive meningococcal disease in Burkina Faso caused by a capsule null locus strain circulating among healthy carriers. *J Infect Dis*. 195:1071-1077

Gharpuray-Pandit D, Coyne J, Eyden B, Banerjee SS
Rhabdomyoblastic differentiation in malignant melanoma in adults: report of 2 cases
International Journal of Surgical Pathology 15, 20-25, 2007

Yamazaki K, Eyden B
Pulmonary lipomatous haemangiopericytoma: report of a rare tumor and comparison with solitary fibrous tumor. *Ultrastructural Pathology* 31: 51-61, 2007

Houreih MA, Eyden BP, Reeve N, Banerjee SS
Aberrant leucocyte common antigen expression in metastatic small cell lung carcinoma: a rare finding and a potential diagnostic pitfall
Applied Immunohistochemistry and Molecular Morphology 15: 236-238, 2007



Programme

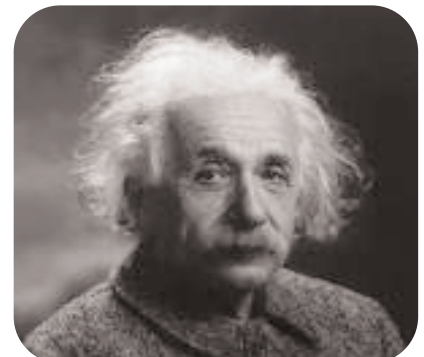
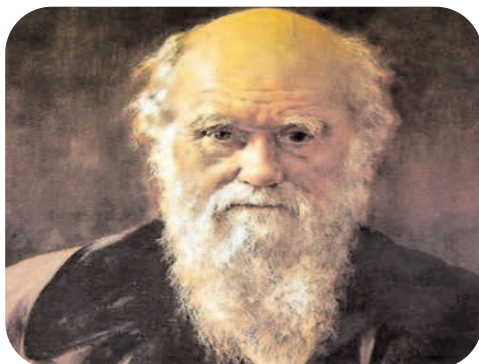
The Society of Electron Microscope Technology

One Day Meeting 12th December 2007

- 9.15am. **Registration, Tea/Coffee, Trade Exhibition**
- 9.55am. **Introduction: Chair, Heather Davies.**
- 10.00am. **Cryo-imaging; its role, application and future at NIBSC**
Roland Fleck, *National Institute of Biological Standards & Control. South Mimms.*
- 10.35am. **Imaging for Television and Publishing**
David Spears, *Clouds Hill Imaging.*
- 11.10am. **Tea, Coffee.**
- 11.30am **Multiple Immunofluorescence Labelling of Formalin Fixed Paraffin Embedded (FFPE)Tissues.**
David Robertson, *Breakthrough, ICR, London.*
- 12.05pm **Investigations using correlative confocal laser scanning microscopy and immunoTEM**
Susan Anderson, *Nottingham University.*
(joint Winner of the Don Claugher Bursary)
- 12.15pm **DC Bursary joint-winner T.B.A.**
- 12.25pm **Buffet Lunch, Trade Exhibition**
- 2.00pm **Electron Microscopy in Paediatric Pathology**
Glenn Anderson, *Histopath. Dept. Great Ormond Street Hospital for Children*
- 2.35pm. **Is there a Future for Electron Microscopy in Healthcare**
Tim Ryder, *EM unit, Charing Cross Hospital.*
- 3.10pm **Tea, Coffee.**
- 3.30pm **Tissue engineering to repair the nervous system and model it in vitro**
James Phillips, *Biological Sciences, Open University.*
- 4.05pm **Carbon nanotube imaging and tracking in vitro and in vivo'**
Prof. Kostas Kostarelos, *School of Pharmacy, London.*
- 4.40pm **Malaria and the red blood cell: mysteries of entrances and exits.**
Prof. Lawrie Bannister, *Sch. Biomedical & Health Sciences, Guy's Campus. KCL*
- 5.15pm **AGM - Wine Reception**
- 7.00pm **Conference Dinner**

To register to attend the conference which is taking place on December 12th at The School of Pharmacy, Brunswick Square, London WC1N 1AX, log on to http://www.semt.org.uk/index_files/Future_Meetings.htm

And now for something completely different!



Members of ACEM who have worked with the ACEM treasurer will know that he is vegetarian (after all, he has to have some faults!). His daughter Amy is also a vegetarian and she receives all kinds of stuff about vegetarianism. Recently she received a list of famous historical figures that were vegetarians – included in this list are the following: Charles Darwin, Albert Einstein, Sir Isaac Newton, Pythagoras, Plato, Socrates, Albert Schweitzer, Nikola Tesla, Leonardo da Vinci, Voltaire, HG Wells etc etc. I'm sure you will agree that this is a very impressive list, but it begs the question, how do they know that these famous people were all vegetarian?