Southampton NHS
University Hospitals NHS Trust

University of Southampton

Postgraduate Short Course in Transmission Electron Microscopy

Aims and Objectives

This 4 day course (limited to 8 students) is intended to train university staff, postgraduate students and biomedical scientists in the understanding and application of transmission electron microscopy (TEM) in the biomedical sciences. Participants are trained in methods of sample preparation and embedding (using their own samples), thin and ultrathin sectioning of resin-embedded samples, the principles and operation of the TEM and presentation of images (including electronic presentation). In addition, participants will acquire the skills to analyse and present data, and be aware of the role, importance and relevance of TEM in biological research. The course is accredited by the Institute of Biomedical Sciences (credit of 27 points).

Lectures

Health & Safety: — 30 mins

Description of general hazards encountered in the laboratory; specific hazards related to electron microscopy; disposal of waste; use of fume hoods.

Choice of fixatives: — 60 mins

Rationale behind fixation procedures for electron microscopy; action of fixatives; use of buffers; osmotic pressure & pH; dehydrating agents; links reagents; resins; block and section stains; examples of appropriate & inappropriate fixation protocols.

Cell structure and resolution: — 60 mins

Definition of resolution; light versus electron microscopy; description of cell structure & examples of cell organelles from a variety of cells and tissues.

TEM design: — 60 mins

Step by step description of the design & function of a transmission electron microscope from the filament down through the lenses and apertures to image recording.

Immunocytochemistry: — 60 mins

Immunolabelling at the TEM level outlining appropriate protocols for pre- & post-embedding labelling; types of fixatives; types of resins; examples of labelled tissue.

Cryosectioning and immunolabelling for TEM: — 60 mins

The theory of the Tokyasu method of ultra thin frozen sectioning and immunolabelling is outlined and a practical protocol is detailed. The rationale for using this approach and some further variations on the basic technique are discussed.

Special techniques, data presentation: — 60 mins

Specialised techniques in TEM; negative staining; freeze fracture; microwave fixation; x-ray microanalysis; a round-up of complimentary microscopies (Scanning electron microscopy, confocal microscopy, time lapse microscopy); presentation of digital images; file types; use of colour; use of image processing packages.

Total Lecture Time: 6 hrs 30 mins

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Practicals

Fixation: Fully supervised step by step fixation of students own specimens from live specimens to infiltration in resin.	4 hrs 30 mins (day one)
Further processing of specimens to resin embedding including labelling of blocks and recording of data.	2 hrs (day two)
Glass knife making	1 hour (day two)
Microtomy: How a microtome works; sectioning of students own polymerised blocks; staining grids.	4 hrs (day three, morning or afternoon)
Microscopy: Use of the transmission electron microscope; viewing stained grids and taking digital photographs.	4 hrs (day three, afternoon or day four morning)
Further supervised use of microtomes and/or microscopes for practice.	4 hrs
Quiz: Image-based quiz showing micrographs of many different types of tissues cells & organelles - both plant and animal; images of tissue fixed using inappropriate protocols; images of sections with faults.	2 hrs
Total Practical Time:	**21 hrs 30 mins**

Reading material

Each course participant is supplied with a booklet containing a range of tissue processing schedules, explanatory notes on the function and operation of the microscope and ancillary equipment, hazard data sheets etc. The BIU library also holds a large number of reference books that are available to the participants.

Assessment

There is no formal assessment but course participants are able to judge their own success by the quality of the images they obtain from their samples. Constant feedback is given throughout the course on ways to improve the various procedures (processing, sectioning, use of the microscope etc.).

Although informal, the end of course quiz is a test of the course participants' ability to recognise a wide range of cell and tissue components at the ultrastructural level - a vital skill in electron microscopy. There are also images of faults in sections and participants learn how to identify and rectify them. Course completion will be certified for participants who are judged by the course organisers to have achieved the course aims by attending the sessions, cutting sections and collecting electron microscope images.

Course Dates and Booking

The course runs approximately once every 6 months and is often over subscribed. To register for a place on the next available course please contact Anton Page (a.page@soton.ac.uk, SGH x4815)