Southampton NHS
University Hospitals NHS Trust

University of Southampton

Postgraduate Short Course in Advanced Light Microscopy

Aims and Objectives

The 5 day course (9 students) is intended to train university staff, postgraduate students and biomedical scientists in the understanding and application of advanced techniques in light microscopy (LM) in the biomedical sciences. participants will be given theoretical and practical training in a variety of LM imaging techniques and then be expected to acquire a portfolio of images from a range of microscopes using both pre-prepared specimens and ones that they prepare themselves. In addition, course participants will acquire skills in analysing, processing and presenting still and video LM data, and be aware of the role, importance and relevance of light microscopy in biological research. The course is accredited by the Institute of Biomedical Sciences (credit of 36 points).

By the end of the course participants should:

- Understand how basic microscopy principles apply to the various types of light microscopes available together with their advantages and limitations.
- Understand fluorescent labeling procedures and how to adapt them for particular applications.
- Have hands on experience of the range of light microscopes available in the Biomedical Imaging Unit.
- Be able to acquire multi-channel images on confocal microscopes.
- Understand the concepts behind some advanced techniques in confocal microscopy.
- Be able to set up and acquire time lapse movies.
- Be able to store and retrieve digital microscope images from a secure university server.
- Understand basic image enhancement and processing procedures.
- Prepare images and movies for power-point presentation to a group.

Lectures

Heath & Safety: — 30 mins

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

Anatomy of a light microscope: — 60 mins

Overview of the basic principles of light microscopy including the properties of light; the concepts of resolution, magnification & contrast; a review of the main optical contrasting techniques

Fluorescence microscopy: — 60 mins

What is fluorescence; excitation and emission spectra, Stoke's shift and emission intensity; epi-illumination; filter cubes and filter types; fluorescence on confocal microscope systems; multiphoton fluorescence; new developments.

Types of fluorescence staining 1: — 60 mins

The advantages and disadvantages of fluorescence labels, what affects their efficacy and how their use can be optimised. Basic application of fluorescence labels in immunocytochemistry and other affinity-cytochemistry techniques.

Types of fluorescence staining 2: — 60 mins
An introduction to the wide range of non-immunological fluorescence staining methods; nuclear counterstains; organellar markers; cell trackers; fluorescent microspheres; viability assays; lipid and membrane probes; ligands and lectins; ion, small molecule and pH probes; in house labelling, genetically engineered fluorescent constructs.

Confocal microscopy: — 60 mins
Explanation of the principles of confocality and the design of a confocal microscope concentrating particularly on our Leica SP2 and SP5 machines; Simultaneous versus sequential image acquisition; appropriate specimens and specimen preparation; examples of applications using a range of fluorochromes separately and in combination.

Live cell imaging: — 60 mins
Why time-resolved microscope imaging is important and how the additional constraints of imaging live biological material can be overcome. Basic requirements for live cell imaging and how the resulting information can be stored and analysed.

Advanced Confocal techniques: — 60 mins
Specialised techniques in confocal microscopy with examples including: Forster resonance energy transfer; fluorescence recovery after photobleaching; fluorescence loss after photobleaching; photoactivatable dyes & calcium imaging.

Workshop, Problems & questions: — 60 mins
Round-table discussion of users' research questions and microscopy applications facilitated by the course organisers.

Image processing: — 90 mins
Post acquisition processing of images and movies and presentation of data; image types and file formats; image compression methods, image quality vs. size, JPEG images; colour space; extended focus methods; movie formats and compression; movie quality vs. file size; importing movies into Powerpoint presentations; exporting data from imaging unit microscopes; introduction to basic Photoshop tools for image processing; practical exercise in creating a composite image and adding a scale bar.

Application seminar: — 60 mins
Presentation of a current research topic that relies heavily on microscopy.

Total: 11 hrs

Practicals

Köhler illumination — 30 mins
How to set up a light microscope.

Viewing specimens Different types of microscopes; dissecting, bright field, phase contrast, DIC, fluorescence & polarising.	3 hrs
Fluorescent labelling of cell cultures.	4 hrs (day two)
Viewing specimens on fluorescent microscopes.	2 hrs (day two)
Use of time lapse and confocal microscopes rotating in three groups of three students.	12 hours (day three & four)
Image processing: Students process the images & movies acquired during the week for presentation to the whole group.	1 hr 30 mins
Micrograph & movie competition: Students present their images and movies to be judged by the rest of the group.	1 hr 30 mins
Total:	**24 hrs 30 mins**

Reading material

Each course participant is supplied with a booklet containing a range of tissue processing protocols and explanatory notes on the function and operation of different types of light microscope. They are also given a booklet entitled "Basics of Light Microscopy & Imaging". The BIU library also holds a large number of reference books that are available to the participants. Additional background material from various microscope suppliers is supplied in PDF format, a list of relevant web resources and tutorials is provided and up to date copies of installers for relevant microscopy and image processing freeware and shareware for PC and Mac are made available.

Assessment

There is no formal assessment but the course participants must produce a range of images and at least one movie per group for entry into the competition on the final afternoon. Prizes are awarded to the winners. Constant feedback is given throughout the course on ways to improve the quality of the images and movies and to discuss practical issues and approaches. Course completion will be certified for participants who are judged by the course organisers to have achieved the course aims by attending the sessions and collecting 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)