CNWY40120 Advanced Biological Imaging

2024

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Module Descriptor

This module is designed for students who wish to understand and become critically aware of the principles, practice and applications of rapidly developing imaging technologies. Particular focus is given to fluorescent light-based contemporary imaging approaches such as confocal microscopy, as well as sample preparation and image analysis. A series of lectures will inform about the concepts of imaging and microscopy; importance of resolution and its limits; optical components; application of histology, immunohistochemistry and immunofluorescence; basics of confocal microscopy, light sheet microscopy, super resolution microscopy, multi-photon microscopy, high content screening microscopy; techniques in light microscopy and live cell imaging; and also correlative light - electron microscopy (CLEM). Complementary workshop sessions will provide context to lectures, allowing students to appreciate the practicalities of specific imaging modalities.

Learning Outcome

On completion of this module students will have extensive insight into the variety of light microscopes available and associated techniques that can be applied in the study biological samples.

Workload

Lectures: 16 h (+ 3 h presentation session)

Laboratories/Workshops: 4 h

Autonomous student learning: 76 h

Assessment

Exam: Mid-semester exam 1h (timing unspecified):	50% (Grade)
Assignment: Presentation:	50% (Grade)

WEEK 9

Monday, November 4th, 10-12, TBC

Lecture 1 - Introduction to biological imaging (DS)

Introduction to module. History. Principles of biological imaging, basic history of imaging and instrumentation. Magnification and resolution, Nyquist criterium, Abbe's formula, basics of optics. Dynamic range, bit depth, scale bars, use of colour.

Lecture 2 - Microscopy components (DS)

Microscope body configurations. Infinity optical system. Aberrations. Objectives, optical elements. Light sources, excitation and emission filters, dichroic mirrors, AOTF, scanners, cameras, PMTs.

Wednesday, November 6th, 10-12, TBC

Lectures 3 - Fluorescence and fluorescent markers (DS)

Principles of fluorescence, basics of fluorescence light microscopy, Jablonski diagrams, fluorescent dyes and chemicals, immunofluorescence, fluorescent proteins, FISH. TIRF microscopy.

Lecture 4 - Confocal microscopy part I (DS)

Principles of confocal microscopy, wide-field versus confocal microscopy, optical sectioning, point scanner and Nipkow disk-based confocality, confocal microscope components. Fundamentals of confocal imaging, system set-up, pixel dwell time, image size, excitation and detection settings, averaging, Airy scanning.

Friday, November 8th, 10-12, TBC

Lecture 5 - Confocal microscopy part II (DS)

Considerations for confocal microscopy of living cells and other moving objects. Effects of confocal setup on cell health and viability. Climate and environmental control during live cell imaging. Multiplexing. Comparison of contemporary confocal microscopes from leading manufacturers. Multi-photon microscopy.

Lecture 6 - Confocal microscopy techniques (DS)

Introduction to cell and protein mobility techniques, live cell imaging, photobleaching, FRAP and photo-activation microscopy. Protein-protein techniques in microscopy, FRET, FLIM, FCS, FCCS, PLA. Slides and culture dishes for microscopy.

Friday, November 8th, 13-16, Conway Imaging core

Workshop 1 – confocal microscopy

WEEK 10

Monday, November 11th, 10-12, TBC

Lectures 7-8 - High content screening microscopy and image analysis (JCS)

Principles of automated microscopy, technologies involved, instrumentation available, limitations, autofocus, image acquisition, cell-based screening. Overview of image processing, basics of image analysis and quantification, tools and software available, deconvolution, image presentation.

Monday, November 11th, 14-17, TBC

Workshop 2. High content screening microscopy (JCS)

Wednesday, November 13th, 10-12, TBC

Lecture 9 – Transmission light microscopy (DS)

Basic principles of bright field imaging, methods of contrast formation and enhancement, Koehler illumination, phase contrast, use of phase rings, DIC, dark field microscopy.

Lecture 10 - Histology, immunohistochemistry, sample preparation (DS)

Applications, fixation methods, tissue processing, embedding, sectioning, staining and stains available, artefacts. Sample preparation for immunohistochemistry, direct and indirect immunohistochemistry, double- and multiple labelling, noise and tools for noise reduction, cryo-sections, paraffin and resin sections, immunofluorescence, slide mounting and storage, dishes for cell culture and live cell imaging.

Wednesday, November 13th, 14-17, Conway Imaging core

Workshop 3 –Transmission light microscopy

WEEK 11

Monday, November 18th, 10-12, TBC

Lecture 11 - Ultrastructural imaging (DS)

Overview of ultrastructural techniques, scanning electron microscopy, transmission electron microscopy, EM instrumentation, basics of sample preparation, cryo-fixation and substitution, chemical fixation.

Lectures 12 - Correlative microscopy (DS)

Overview of correlative light-electron microscopy (CLEM) techniques, basics of sample preparation, CLEM applications and image analysis.

Wednesday, November 20th, 10-12, TBC

Lectures 13 - Advanced light microscopy technologies (DS).

Principles and applications of light sheet (LS) microscopy, sample preparation by expansion.

Lectures 14 - Image analysis (DS)

Segmentation, measurements, statistics, 3D-rendering: ImageJ/FIJI, Imaris, ilastic, etc.

Friday, November 22nd, 10-12, TBC

Lectures 15 - Super-resolution microscopy (DS)

AiryScan, Structured Illumination Microscopy (SIM), Super-Resolution Radial Fluctuations

SRRF.

Lectures 16 - Super-resolution microscopy (DS)

Stimulated Depletion Microscopy (STED), Single molecule localization microscopy (STORM, PALM, PAINT, etc.), MinFlux.

Friday, November 22nd, 14-17, Conway Imaging core

Workshop 4 – SEM

TBCth December, TBC. Mid-term exam

TBC

Assessment - Presentations (DS and JCS)

Students will be required to give a short presentation on a microscopy-related topic to the group.