

CNWX40120 Advanced Biological Imaging

2017

Prof. Dimitri Scholz (DS) & Prof. Jeremy C. Simpson (JCS)

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 transmission and fluorescent light-based imaging approaches. 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 the relationship between light microscopy and electron microscopy. 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: 13 h (+ 3 h presentation session)

Laboratories/Workshops: 12 h

Autonomous student learning: 76 h

Assessment

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

Assignment: Presentation: 50% (Grade)

WEEK 1

Tuesday 14th November, 10-12, Conway Imaging facility

Lecture 1 - Introduction to biological imaging (DS)

Introduction to module. Principles of biological imaging, basic history of imaging and instrumentation, temporal resolution, Nyquist, Abbe, basics of optics. Understanding of what makes an image, importance of bit depth, scale bars, use of colour.

Lecture 2 - Microscopy components (DS)

Microscope body configurations, objectives, optical elements, light sources, lasers, excitation and emission filters, dichroic mirrors, AOTFs, scanners, detectors, cameras, PMTs.

Thursday 16th November, 10-12, Conway Imaging facility

Lecture 3 - Bright field microscopy (DS)

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

Lecture 4 – 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, immunolabelling, slide mounting and storage.

Thursday 16th November, 14:00-17:00, Conway Imaging facility

Workshop 1 – online tutorials (DS)

WEEK 2

Tuesday 21st November, 10-12, Conway Imaging facility

Lecture 5-6 - Ultrastructural imaging I (DS)

Overview of ultrastructural techniques, scanning electron microscopy, transmission electron microscopy, EM instrumentation, basics of sample preparation, cryo-fixation and substitution, chemical fixation. Overview of correlative light-electron microscopy (CLEM) techniques, basics of sample preparation, CLEM applications and examples.

Wednesday 22nd November, 10-12, Conway Imaging facility

Lecture 7 - Fluorescence and fluorescent markers (DS)

Principles of fluorescence, Jablonski diagrams, fluorescent dyes and chemicals, immunofluorescence using cultured cells, GFP and variants, DsRed and variants, basics of fluorescence light microscopy.

Wednesday 22nd November, 14:00-17:00, Conway Imaging facility

Workshop 2 – online tutorials (DS)

Thursday 23rd November, 10-12, TBC

Lecture 8 - Confocal microscopy part I (JCS)

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, Kalman averaging.

Lecture 9 - Confocal microscopy part II (JCS)

Considerations for confocal microscopy of living cells. Effects of confocal setup on cell health and viability. Climate and environmental control during live cell imaging.

Friday 24th November, 10-12, TBC

Lecture 10 - Confocal microscopy techniques (JCS)

Introduction to cell and protein mobility techniques, live cell imaging, photobleaching, FRAP and FLIP. Introduction to protein-protein techniques in microscopy, FRET, FLIM, FCS, and FCCS.

Lecture 11 - 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.

Thursday 23rd November 13:00-15:00, Science Centre West 259

Workshop 3 - High content screening microscopy (JCS)

WEEK 3

Tuesday 28th November, 10-12, Conway Imaging facility

Lecture 12-13 - Advanced light microscopy technologies (DS)

How to improve resolution in fluorescence microscopy, super-resolution, illumination based approaches (structured illumination (SI)), acquisition based approaches (STED, PALM, STORM). Principles of light sheet (LS) microscopy, principles of multi-photon microscopy, principles of TIRF microscopy. Proximity ligation assay.

Tuesday 28th November, 14-17 Conway Imaging facility

Workshop 4 – online tutorials (DS)

Thursday 30th November, TBC. Mid-term exam

Monday 4th December, 14-17:00 Venue TBC

Assessment - Presentations (DS and JCS)

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