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Light Microscopy

Semester 1 / Autumn **10 Credits**

Each Course is composed of Modules & Activities.

Modules:

Introduction to Light Microscopy	IMSc
Brightfield and widefield microscopy	IMSc
Confocal microscopy	IMSc
Samples for light microscopy	IMSc
Camera detectors	IMSc
Advanced light microscopy techniques	IMSc
Image formation	IMSc

Each Module is composed of Lectures, Reading Lists, MCQ self-assessments, & Discussion Boards.

These Modules are taught on the following Programmes, or are incorporated into blended Courses which teach students enrolled outwith the Edinburgh Imaging Academy:

- IMSc - Imaging programme

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Modules include:

Introduction to Light Microscopy:
- Introduction to light microscopy
- Behaviour of Light
- Interaction of Light with Matter – Diffraction and Optical Resolution

Brightfield and widefield microscopy:
- Light microscopy & associated techniques
- Determinants of optical resolution & contrast in the bright-field microscope
- Optical contrast techniques
- The epifluorescence microscope

Confocal microscopy:
- Confocality & confocal microscopy methods
- Confocal microscopy – Components 1
- Confocal microscopy – Components 2
- Image acquisition parameters

Samples for light microscopy:
- Sample preparation for light microscopy
- Sample labelling
- Live specimen imaging
- Optimisation of image acquisition

Camera detectors:
- Scientific digital cameras
- Scientific camera control

Advanced light microscopy techniques:
- Multi-photon excitation-based imaging
- Advanced microscopy technologies
- Spectral un-mixing
- Total internal reflection microscopy

Image formation:
- Image formation in microscopy

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Introduction to Light Microscopy

Lecture 1
Title: Introduction to light microscopy
Description: History of microscopy; microscopy techniques; methods to improve contrast and resolution
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Give a general introduction to light microscopy
- Describe techniques now indispensable in modern biomedical research
- Highlight light microscopy importance for life sciences
- Explain why light microscopy has become the most widely used range of imaging technologies in life sciences

Lecture 2
Title: Behaviour of Light
Description: The nature and duality of light
Author(s): Dr Trudi Gillespie
Learning Objectives
- Explain why we use microscopes
- Describe what is required to form & collect an image
- Outline the nature of light in relation to energy & the transportation of energy by electromagnetic waves
- Identify relevant features of the light spectrum
- State key discoveries about the particle & wave behaviour of light
- Describe the photoelectric effect
- Discuss Albert Einstein's theory of the wave-particle duality of light

Lecture 3
Title: Interaction of Light with Matter - Diffraction and Optical Resolution
Description: Diffraction and Optical Resolution
Author(s): Dr. Trudi Gillespie
Learning Objectives
Discuss wave-front propagation & wave-front geometry
- Explain diffraction
- i.e. bending of light encountering an object or a gap
- State what the effect of gap size is (with respect to wavelength) on the spreading of light
- Describe constructive & destructive interference
- State Abbe’s theory of image formation
- Discuss the optical resolution limit imposed by the diffraction of light

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Brightfield and widefield microscopy

Lecture 1
Title: Light microscopy & associated techniques
Description: Practical aspects of the brightfield microscope; Köhler illumination
Author(s): Prof Andrew Jarman
Learning Objectives
- Describe the practical aspects of the brightfield microscope
- Explain the importance of optimal illumination in brightfield microscopy

Lecture 2
Title: Determinants of optical resolution & contrast in the bright-field microscope
Description: Optical resolution; objective numerical aperture; aperture diaphragm
Author(s): Prof Andrew Jarman
Learning Objectives
- Explain magnification, contrast and resolution
- Describe different modes by which light interacts with objects, in particular diffraction
- Explain the role of numerical aperture in imaging
- Demonstrate the condenser's role in image formation

Lecture 3
Title: Optical contrast techniques
Description: Dark-field microscopy; phase contrast microscopy; differential interference contrast microscopy
Author(s): Prof Andrew Jarman
Learning Objectives
- Explain contrast techniques
- Describe the configuration of a dark-field microscope
- State how to obtain contrast from phase changes
- Apply these concepts to:
    - Phase contrast microscopy
    - Differential interference contrast microscopy

Lecture 4
Title: The epifluorescence microscope
Description: Fluorescent markers / tags in biological research
Author(s): Prof Andrew Jarman
Learning Objectives
- Describe the epifluorescence microscope workings
- Explain how brightfield microscopy applies to fluorescence microscopy, including:
    - Resolution
    - Optical aberrations
    - Correction
- State main issues specific to fluorescence microscopy

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Confocal microscopy

Lecture 1
Title: Confocality & confocal microscopy methods
Description: General principles
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Describe the principle of confocal microscopy
- Discuss the function of the basic components of a CLSM
- Explain imaging parameters and how to optimise them

Lecture 2
Title: Confocal microscopy – Components 1
Description: Basic components of a confocal microscopy system
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Explain the first set of important components of a confocal laser scanning microscope:
    - Lasers as excitation light sources
    - Beam splitters as filtering devices that separate excitation from emitted light
    - Objective lenses as the central optical components for image magnification

Lecture 3
Title: Confocal microscopy – Components 2
Description: Basic components of a confocal microscopy system
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Describe a second range of essential components of a confocal laser scanning microscope
- Explain the most important functional properties and their impact on image acquisition
- Summarise the function of a range of essential technical components of a laser scanning microscope

Lecture 4
Title: Image acquisition parameters
Description: Optimisation of imaging parameters for confocal laser scanning microscopy
Author(s): Dr Rolly U Wiegand
Learning Objectives
- Explain how to optimise image acquisition on a CLSM
- Describe the following parameters:
    - imaging by optical sectioning
    - Scan speed/dwell time
    - Image resolution and pixel dimensions
    - Image formation and dynamic range
    - Signal conversion/sampling rate
    - Zoom, S/N ratio, scan averaging
    - Spectral cross-talk and how to avoid it
    - Image display modes

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Samples for light microscopy

Lecture 1
Title: Sample preparation for light microscopy
Description: Specimen preparation and labelling for light microscopy applications
Author(s): Rolly U. Wiegand
Learning Objectives
- Explain the importance of sample preparation and the impact this has on image quality
- Describe the first steps of sample preparation in particular specimen fixation in detail
- Describe appropriate fixation protocols as the first step of immunofluorescence labelling

Lecture 2
Title: Sample labelling
Description: Preparation of fixed and labelled samples for light microscopy
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Explain the steps of sample preparation after the specimens have been fixed.
- Describe the labelling of proteins using antibody-based fluorescence tagging
- Give an overview of all other steps to complete the processing and to mount the sample, now ready for image acquisition

Lecture 3
Title: Live specimen imaging
Description: Fluorescent labelling and specimen maintenance for live specimen imaging
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Explain the main two groups of fluorescent labelling for live specimens
- Describe the experimental environment that needs to be controlled during live cell imaging
- Interpret microscopy setups for live specimen imaging

Lecture 4
Title: Optimisation of image acquisition
Description: Image acquisition parameters
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Describe the most important parameters for image acquisition focussing on live specimen imaging, including:
    - the setting of light sources
    - correct emission filtering and detector adjustment
    - how to save files
    - Explain the importance of correct emission filtering
- Interpret how to save acquired images

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Camera detectors

Lecture 1
Title: Scientific digital cameras
Description: Basics, including sensor architecture, binning, and colour cameras
Author(s): Dr Chris Wood
Learning Objectives
- Give a basic overview description of scientific digital cameras
- Describe camera sensor architecture
- Explain the principle of camera binning
- Highlight the different properties of colour cameras

Lecture 2
Title: Scientific camera control
Description: Sensitivity & spectral response, noise, SNR, camera gain, camera advantages and considerations, well depth & dynamic range, and camera speed
Author(s): Dr Chris Wood
Learning Objectives
- Describe camera sensitivity and spectral response
- List sources of noise
- Explain how to calculate signal to noise ratios
- Define camera gain state some practical aspects of choosing the right gain
- Define well depth and dynamic range
- State existing camera speeds
- Discuss camera advantages and considerations

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Advanced light microscopy techniques

Lecture 1
Title: Multi-photon excitation-based imaging
Description: Elastic scattering, multi-photon excitation, basic microscope set-ups
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Explain elastic scattering in biological tissues
- Interpret multi-photon excitation of standard fluorophores
- Describe basic microscope set-ups for intra-vital imaging

Lecture 2
Title: Advanced microscopy technologies
Description: Förster resonance energy transfer; measurement of inter-molecular interactions; fluorescence lifetime imaging; time-correlated single photon counting;
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Interpret:
    - Förster resonance energy transfer
    - Light microscopy-based measurement of inter-molecular interactions
    - Fluorescence lifetime imagingT
    - ime-correlated single photon counting

Lecture 3
Title: Spectral un-mixing
Description: Basics of spectral un-mixing, the spectral cross-talk problem …
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Explain the spectral cross-talk problem
- Interpret an alternative spectral separation technology – un-mixing
- Compare briefly different microscope set-ups for spectral un-mixing
- Explain the basics of spectral un-mixing
- Give an example of how to eliminate auto-fluorescence background from an image

Lecture 4
Title: Total internal reflection microscopy
Description: Evanescent field TIRF microscope; experimental purposes
Author(s): Dr. Rolly U. Wiegand
Learning Objectives
- Describe the principle of generating an evanescent field
- Interpret how this principle is integrated in a TIRF microscope
- Explain how this specialised technology can be used for experimental purposes

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Image formation

Lecture 1
Title: Image formation in microscopy
Description: Resolution & causes of image degradation
Author(s): Dr Trudi Gillespie
Learning Objectives

- State Abbe's theory of image formation
- Explain the concepts of image quality, image degradation & image restoration
- List some sources of image degradation
- Describe the model of image blur – the point spread function
- Explain the relationship between optical resolution and the point spread function
- Interpret the three-dimensional point spread function & axial resolution