Micron OXFORD Advanced Bioimaging Unit

UNIVERSITY OF OXFORD

Department of Biochemistry

wellcome trust Strategic Award

Micron Advanced Microscopy Course

16th-20th November 2020, Virtual course

Course Structure

Monday 16th Nov	Tuesday 17th Nov	Wednesday 18th Nov	Thursday 19th Nov	Friday 20th Nov
Principles of light microscopy	Fluorescence microscopy	Quantitative bioimaging	Super-resolution microscopy	Demos and Practicals

Course organisers

Dr Carina Mónico & Dr Nadia Halidi

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Course Programme and Schedule

Day 1: Monday 16th November 2020: Principles of Light Microscopy		
Morning session: Introduction to light microscopy and microscope components Chair: Alan Wainman		
Time	**Lecture**	**Speaker**
09:00 – 09:15	Welcome to the course	Ilan Davis
09:20 – 09:50	Introduction to light microscopy	Nadia Halidi
09:55 – 10:55	Basic optics for microscopy, image formation	Ian Dobbie
10:55 – 11:15	**Break**	
11:15 – 11:45	Anatomy of a microscope	Carina Mónico
11:50 – 12:35	Brightfield techniques, Contrast enhancement	Ian Dobbie
12:35 – 13:35	**Lunch**	
Afternoon session: Microscope components Chair: Nadia Halidi		
13:35 – 14:35	Objectives, Optical Aberrations, Resolution, Point Spread Function	Chris Lagerholm
14:35 – 14:55	**Break**	
14:55 – 15:40	Cameras for Imaging	Photometrics
15:45 – 16:30	Detectors for Imaging	Zeiss
16:30 – 17:00	Panel discussion, wrap-up and quiz	All speakers

Day 2: Tuesday 17th November 2020: Fluorescence Microscopy		
Morning session: Introduction to Fluorescence Microscopy and fluorescent probes Chair: Ian Dobbie		
Time	**Lecture**	**Speaker**
09:00 – 09:30	Demo on microscope anatomy and setting Köhler illumination	Olympus
09:35 – 10:30	Introduction to fluorescence microscopy	Carina Mónico
10:30 – 10:50	**Break**	
10:50 – 11:40	Fluorescent dyes and proteins	Mark Howarth
11:45 – 12:30	Sample preparation – practical considerations	Lothar Schermelleh
12:30 – 13:30	**Lunch**	
Afternoon session: Fluorescence imaging of cells and tissues. Chair: Carina Mónico		
13:30 – 14:30	Confocal microscopy and optical sectioning	Alan Wainman
14:35 – 15:05	Two – photon microscopy	Emily Thornton
15:05 – 15:25	**Break**	
15:25 – 16:10	Lightsheet microscopy	Matthew Stower
16:10 – 17:00	Panel discussion, wrap-up and quiz	All speakers

Day 3: Wednesday 18th November 2020: Quantitative bioimaging		
Morning session: Quantitative imaging. Chair: Lothar Schermelleh		
Time	**Speaker**	**Lecture**
09:00 – 09:45	Live – cell imaging	Nadia Halidi
09:50 – 10:50	F-techniques: Measuring molecular motion and interactions	Chris Lagerholm
10:50 – 11:10	**Break**	
11:10 – 12:10	Biological electron microscopy: techniques and applications	Errin Johnson
12:10 – 13:10	**Lunch**	
Afternoon session: Image data handling and analysis. Chair: Nadia Halidi		
13:10 – 13:55	Basic image processing, data handling and storage	David Pinto
14:00 – 15:00	Introduction to image analysis	Ulrike Schulze
15:00 – 15:20	**Break**	
15:20 – 15:50	Artificial intelligence and machine learning in microscopy	Dominic Waithe
15:50 – 17:00	Panel discussion, wrap-up and quiz	All speakers

Day 4: Thursday 19th November 2020: Super-resolution microscopy		
Morning session: Structure Illumination and localisation microscopy Chair: Ian Dobbie		
Time	**Lecture**	**Speaker**
09:00 – 10:30	Introduction to Super-resolution. Structured Illumination microscopy	Lothar Schermelleh
10:30 – 10:50	**Break**	
10:50– 12:05	Single-molecule imaging and localisation microscopy	Stephan Uphoff
12:05– 13:00	**Lunch**	
Afternoon session: Nanoscopy and Custom-built systems Chair: Carina Mónico		
13:00 – 13:45	STimulated Emission Depletion microscopy (STED)	Silvia Galiani
13:50 – 14:50	Introduction to Bespoke Microscope Design	Ian Dobbie
14:50 – 15:10	**Break**	
15:10 – 17:00	Panel discussion, wrap-up and quiz. Discussion on the different microscopy techniques. Students imaging challenges.	All speakers

Day 5: Friday 20th November 2020: Demos and Practicals		
Morning session: Conventional imaging Chair: Dalia Gala		
Time	**System Demo**	**Demonstrator**
09:00 – 09:25	Widefield fluorescence imaging and deconvolution (Deltavision Elite)	Ian Dobbie
09:30 – 9:55	Spinning disk confocal (PerkinElmer UltraVIEW)	Alan Wainman
10:00 – 10:25	Laser scanning confocal (Olympus FV3000)	Carina Mónico
10:25 – 10:45	**Break**	
10:45 – 11:10	Laser scanning confocal with Airyscan detector (Zeiss LSM 980)	Nadia Halidi
11:15 – 11.40	Lightsheet microscope (Zeiss Z1)	Matthew Stower
11.45 – 12:10	Image analysis demo on Fiji	Urlike Schulze
12:10– 13:00	**Lunch**	
Afternoon session: Super-resolution and custom-built systems Chair: Darragh Ennis		
13:00 – 13:25	Single-molecule imaging, STORM (Nanoimager)	Sandip Kumar
13:30 – 13:55	STED (Leica TCS SP8)	Silvia Galiani
14:00 – 14:25	4Pi (Bespoke)	Jingyu Wang
14:25 – 14:45	**Break**	
14:45 – 15:10	Structured Illumination Microscopy (OMX – V3)	Lothar Schermelleh
15.15 – 15:40	TIRF – SIM (Bespoke)	Kseniya Korobchevskaya/ Marco Fritzsche
15:45 – 16:10	Deep SIM (Bespoke)	Nick Hall

Detailed course programme

Day 1: Mon 16 Nov 2020 - Principles of Light Microscopy

Morning session: Introduction to light microscopy and microscope components (Chair: Alan Wainman)

- Course and logistics announcements

1. **Welcome to the course** Ilan Davis
   - Introduction to the course, goals, motivation
   - Overview of the Micron Advance Bioimaging Unit and the Oxford Bioimaging community

2. **Introduction to light microscopy** Nadia Halidi
   - What is microscopy?
   - Bioimaging workflow: From sample preparation, imaging to data management, analysis and visualization
   - What is important for good microscopy? Contrast. Resolution.
   - Limitations of light microscopy

3. **Basic optics for microscopy, image formation** Ian Dobbie
   - Nature of light
   - Basic understanding of lenses, refraction and diffraction
   - Constructive/ destructive interference
   - Image formation

4. **Anatomy of a microscope** Carina Mónico
   - Types of microscopes, inverted, upright, stereoscopes
   - Anatomy of a microscope
   - Understanding conjugate planes

5. **Brightfield techniques, Contrast enhancement** Ian Dobbie
   - What is brightfield and when it is used
   - Köhler illumination
   - Overview of increased contrast in BF (Dark field, PhC, mention DIC)

Afternoon session: Microscope components (Chair: Nadia Halidi)

6. **Objectives, Optical aberrations, Resolution, Point Spread Function** Christopher Lagerholm
   - Objective lenses properties, Numerical Aperture, Working distance, immersion media
   - Understanding of factors affecting image quality, refractive index mismatch, coverslip thickness, aberrations
   - Axial and lateral resolution, PSF, Airy, Abbe, Rayleigh, Sparrow

7. **Cameras for Imaging** Photometrics
   - Different camera technologies and how they work
   - Sensitivity, Quantum efficiency, Noise, Nyquist sampling
   - Compare different cameras

8. **Detectors for Imaging** Zeiss
   - Different detectors and how they work (including airyscan 2 and non-descanned detectors)
   - Compare different detectors; Sensitivity, Quantum efficiency, Noise, Nyquist sampling

9. **Panel discussion, wrap-up session and Quiz** All speakers
   - Quiz
   - Guided discussion through topics of the day that need revisiting

Day 2: Tue 17 Nov 2020 - Fluorescence Microscopy

Morning session: Introduction to Fluorescence Microscopy and fluorescent probes (Chair: Ian Dobbie)

10. Demo on microscope anatomy and setting Köhler illumination

Micron or Olympus

- Anatomy of an inverted, upright and stereoscopes microscope
- Conjugate planes on a microscope
- Setting Köhler illumination

11. Introduction to fluorescence microscopy Carina Mónico

- Why Fluorescence? Contrast.
- What is fluorescence? Simplified Jablonski diagram
- Absorption and emission spectra, Stokes shift, crosstalk
- Basic principle and components of a fluorescence microscope
- Transmitted vs. Reflected light
- Fluorescent light sources
- Fluorescence filter sets: emission, excitation, dichroic, polychroic
- Micron SPEKcheck tool
- Widefield fluorescence microscopy: what it is and which samples are suited
- Capabilities of current widefield systems (timelapse, multi-point visiting, multi-area)
- Interactive session on setting filter sets
- OTF and Deconvolution

12. Fluorescent dyes and proteins Mark Howarth

- Jablonski diagram
- Key characteristics of a fluorophore: quantum yield, photostability, brightness
- Organic and inorganic fluorophores
- Antibody targeting and how to label protein with dye; direct / indirect labelling
- Site-specific protein labeling methods (SNAP-tag etc.)
- Labeling different organelles
- Different fluorescent proteins- advantages and concerns

13. Sample preparation - tips and tricks Lothar Schermelleh

- Fixed vs. Live
- Sample mounting

- Typical immunocytochemistry protocol (fixation, permeabilization, blocking, washes immunostaining, mounting)
- Troubleshooting
- Good staining controls

Afternoon session: Fluorescence imaging of cells and tissues (Chair: Carina Mónico)

14. Confocal microscopy and optical sectioning Alan Wainman

- History
- Pinhole blocks out-of-focus light
- Optical sectioning
- Point scanning confocal light path and components
- Setting offset and gain
- Bleed through and sequential scanning
- Spectral unmixing
- Spinning Disk Microscopes
- Comparison of Scanning Confocal vs. Spinning Disk vs. Widefield
- TIRF microscopy

15. Two-photon microscopy Emily Thornton

- 2-photon vs. 1-photon excitation
- Limits of light penetration in deep tissue: scattering, absorption
- 2-photon vs. confocal
- Instrumentation and lasers for 2 photon microscope
- Benefits of using non-descaned detectors in multi-photon microscopy
- Advantages of 2-photon microscopy and applications

16. Lightsheet microscopy Matthew Stower

- Problems of imaging deep
- Lightsheet microscopy and optics
- Sample preparation (live samples, Tissue Clearing)
- Drawbacks and Artifacts
- Light Sheet Implementations (variations of SPIM or LSFM)
- Biological application example: light-sheet imaging to study development

17. Panel discussion, wrap-up session and Quiz All speakers

- Quiz
- Guided discussion through topics of the day that need revisiting

Day 3: Wed 18 Nov 2020 - Quantitative imaging

Morning session: Quantitative imaging (Chair: Lothar Schermelleh)

18. Live-cell Imaging Nadia Halidi

- Why live? Comparison of live cell imaging and fixed cell studies.
- Requirements for live imaging: sample preparation (mounting, staining, media), choice of equipment and additional components to maintain cell viability.
- Efficiency of detection: objectives, filter sets, detectors
- Photobleaching, phototoxicity & O_2 scavenging systems

19. F-techniques: Measuring molecular motion and interactions Chris Lagerholm

- Fluorescence techniques for molecular dynamics; FRAP, FLIP, FLAP, FCS, FLIM
- Techniques to measure molecular interactions such as FRET and FCCS

20. Biological electron microscopy: Biological electron microscopy: techniques and applications Errin Johnson

- Basic principles of electron microscopy and applications to biological research
- Biological specimen preparation for EM
- Advanced EM techniques: cryo-EM, EDS x-ray microanalysis, correlative light & EM, 3D-EM

Afternoon session: Image data handling and analysis (Chair: Nadia Halidi)

21. Basic image processing, data handling, storage David Pinto

- What is a digital image?
- What makes a good image?
- Options and likely current candidates for data storage after acquisition
- Pitfalls/Advantages of many approaches including annotation, de-duplication, backup, sharing and searching for data
- OMERO approach to solving the above
- Figure preparation guidelines for publication

22. Introduction to image analysis Ulrike Schulze

- Design of experiments to achieve a specific measurement goal
- Quick summary of available image processing & analysis software

- Greyscale, LUT, Composites, RGB, Stacks
- Filtering images to enhance features of interest
- Image segmentation
- Deconvolution
- Co-localisation statistics
- 3D visualisation & analysis
- Automation of processing and analysis tasks

23. Artificial intelligence and machine learning in microscopy
Dominic Waithe

- Overview of AI and introduction to machine learning (ML)
- ML applied to Microscopy
- Practical considerations for ML

24. Panel discussion, wrap-up session and Quiz All speakers

- Quiz
- Guided discussion through topics of the day that need revisiting

Day 4: Thu 19 Nov 2020 - Super-resolution microscopy

Morning session: Structured Illumination and localisation microscopy (Chair: Ian Dobbie)

25. Introduction to Super-resolution / Structured Illumination Microscopy Lothar Schermelleh
    - Brief Need for super res and history and Overview of super resolution
    - Principles of SIM
    - Linear SIM methods
    - Reconstruction artifacts
    - Lattice SIM
    - Biological applications
    - Pros and cons

26. Single-molecule imaging and localisation microscopy Stephan Uphoff
    - The principle of localisation microscopy: imaging individual molecules
    - TIRF Microscopy
    - Single-molecule imaging
    - Variants of LM: PALM, FPALM, STORM, dSTORM, GSDIM
    - Tips on sample preparation for Localisation microscopy
    - Biological applications
    - Pros and cons

Afternoon session: Nanoscopy and custom-built systems (Chair: Carina Mónico)

27. STimulated Emission Depletion microscopy (STED) Silvia Galiani
    - Principles of STED, RESOLFT
    - Sample preparation & dyes
    - Biological applications
    - Challenges of STED microscopy
    - Minflux microscopy

28. Introduction to Bespoke Microscope Design Ian Dobbie
    - Why build a microscope? Example DeepSIM
    - Design and built considerations
    - Adaptive Optics
    - CryoSIM
    - Maintenance and sustainability

29. Students imaging challenges. Conclusions and panel discussion on different microscopy techniques All speakers

- Quiz
- Practical considerations to choose the right microscopy technique

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