# LIGHT MICROSCOPY PHD COURSE

# PROGRAMME

## PRINCIPLES OF MICROSCOPY

12.08.18-16.08.2019

## CONFOCAL AND FLUORESCENCE MICROSCOPY

26.08.18-30.08.2019

University of Copenhagen, Department of Biomedical Sciences
Core Facility for Integrated Microscopy
*in Collaboration with* The Royal Microscopical Society

| Monday 12 August | | |
|---|---|---|
| **09.00 – 09.30** | *Introduction* | KQ/CP |
| **09.30 – 10.15** | *Lecture*<br>The story of the microscope | PJE |
| **10.15** | **Coffee** | 15.2 |
| **10.30 – 11.30** | **Lecture**<br>Limitations of the eye. Resolution, contrast, magnification.<br>Lenses, magnifying glasses, compound microscopes. | PJE |
| **11.30 – 11.45** | **Break** | 15.2 |
| **11.45 – 12.45** | **Lecture**<br>Conjugate planes | PJE |
| **12.45** | **Lunch** | |
| **13.30 – 14.15** | ***Lecture***<br>Köhler illumination | PJE |
| **14.15 – 15.00** | ***Practical 1 (rotation 1)***<br>Köhler illumination (4) — CP<br>Conjugate planes on the optical bench (3) — AS<br>Conjugate planes in the microscope (3) — PJE<br>Workbook DIY (1 – 4) — THB/PHV | |
| **15.00** | **Coffee** | 15.2 |
| **15.15 – 16.45** | ***Practical 1 (rotations 2 and 3)*** | |
| **16.45 – 17.00** | **Summary of day's work** | |

**You should now understand the geometrical optics of the microscope, know how to set it up, and begin to understand why these steps are necessary.**

| Tuesday 13 August | | |
|---|---|---|
| **09.00 – 09.45** | *Practical 1 (rotation 4)* | |
| **09.45** | **Coffee** | **15.2** |
| **10.00 – 11.00** | *Lecture* | |
| | Lens defects and their correction | PJE |
| **11.00 – 11.05** | Short break | |
| **11.05 – 11.30** | *Demonstration* | |
| | Setting up Köhler illumination in transmitted light | PJE |
| | Depth of field and depth of focus | |
| **11.30 – 12.30** | *Lecture-demonstration* | |
| | Diffraction, resolution and contrast | PJE |
| **12.30** | **Lunch** | |
| **13.15 – 14.00** | *Lecture-demonstration continued (video)* | PJE |
| **14.00 – 14.45** | *Practical 2 (rotation 1)* | |
| | ▪ Diffraction experiments(6)<br>▪ Aperture (7)<br>▪ Resolving power (8)<br>▪ Work Book DIY (1-8) | PJE<br>AS<br>CP<br>THB/PHV |
| **14.45** | **Coffee** | **15.2** |
| **15.00 – 15.45** | *Practical 2 (rotation 2)* | |
| **15.45 – 16.30** | **Summary of day's work** | |

**You should now understand how diffraction sets the limits to resolving power, and provides the basis for generation of contrast.**

| Wednesday 14 August | | |
|---|---|---|
| **09.00 – 09.45** | ***Practical** 2 (rotation 3)* | |
| **09.45 - 10.30** | ***Practical** 2 (rotation 4)* | |
| **10.30** | **Coffee** | **15.2** |
| **10.45 – 11.30** | ***Lecture***<br>Contrast. Bright field, dark ground, Rheinberg,<br>Phase contrast | PJE |
| **11.30 – 12.15** | ***Practical** 3*<br>Dark field – patch stop (9)<br>Rheinberg (10) | |
| **12.15- 13.00** | **Lunch** | |
| **13.00 – 13.30** | ***Lecture***<br>The nature and properties of light | AS |
| **13.30** | **Coffee** | **15.2** |
| **13.45 – 14.45** | Equations for limit of resolution of optical instruments | AS |
| **15.00 – 16.30** | ***Practical 4***<br>Phase contrast (11) | |

**You should now understand how the properties of specimens may be exploited in the microscope to give rise to contrast.**

| Thursday 15 August | | |
|---|---|---|
| **09.00 – 10.00** | ***Lecture-demonstration***<br>Polarised light | AS |
| **10.00** | **Coffee** | **15.2** |
| **10.15 – 11.30** | ***Practical 5***<br>Contrast in the polarised-light microscope (13)<br>Effects of mounting media | |
| **11.30 – 12.00** | ***Lecture***<br>*Understanding interference colours* | AS |
| **12.00** | **Lunch** | |
| **12.45 – 13.15** | ***Lecture***<br>Differential interference contrast | PJE |
| **13.15 – 14.15** | *Practical 6 (rotation 1 and 2)*<br>▪ Polarised light. examples at lightbox (12-13)<br>▪ DIC (Epi-illumination and transmitted light) (14)<br>▪ DIC on a Laser Scanning Microscope (15)<br>▪ Workbook (continue + 16) | <br>AS<br>PJE<br>CP<br>THB/PHV |
| **14.15** | **Coffee** | **15.2** |
| **14.30 – 15.30** | *Practical 6 (rotation 3 and 4)* | |
| **15.30 – 16.15** | *Lecture*<br>Methods of recording images and fitting the camera to a microscope | PJE |
| **16.15 – 16.30** | **Summary of day's work** | |

**You should now understand the concept of optical path difference and how polarisation colours arise, and how these can be applied to generate contrast in the microscope image.**

| Friday 16 August | | |
|---|---|---|
| **9.00 – 9.15** | *Lecture*<br>Stereomicroscopes | PJE |
| **09.15 – 10.00** | *Lecture*<br>Principles of fluorescence and confocal microscope | PJE |
| **10.00** | **Coffee** | **CFIM** |
| **10.15 – 11.45** | **Practical 7 ( Rotation 1 and 2)** | **CFIM** |
| | Maintenance and cleaning of a microscope (18) and Alignment of the Hg arc (19) | CP |
| | Introduction to fluorescence microscopy | THB/PHV |
| | Introduction to fluorescence microscopy | |
| | Intro to scanning and Transmission electron microscopy | KQ |
| **11.45** | **Lunch** | |
| **12.30 – 14.00** | ***Practical 7 ( Rotation 3 and 4)*** | |
| **14.00** | **Coffee** | **15.2** |
| **14.15 – 15.15** | *Lecture*<br>Sample preparation – practical considerations | **15.2**<br>CP |
| **15.15 – 15.45** | **Questions; summary and evaluation of course** | |

**You should now know the principles of light microscopy**
**See you in a week!!**

| Monday 26 August | | |
|---|---|---|
| **9.00 – 09.45** | *Lecture*<br>Intro to Fluorescence | CP |
| **09.45 – 10.30** | *Lecture*<br>Confocal Microscopy | CP |
| **10.30** | **Coffee** | 15.2 |
| **10.45 – 12.15** | *Lectures*<br>Confocal microscopy (cont)<br>Introduction to ZEN software<br>Digital imaging | CP<br>CP<br>CP |
| **12.15** | **Lunch** | |
| **13.00 – 14.15** | *Lecture- Remote session*<br>Digital imaging, imaging dimensions | CP |
| **14.015** | **Coffee** | CFIM |
| **14.30 – 16.30** | **Practical 1 ( groups 1-3)**<br>Single point laser scanning microscopy<br>• Channel design<br>• Bleed through/cross-excitation<br>• Dynamic range/ SN ratio<br>• Digital resolution | CFIM |
| **16.30- 17.00** | **Summary of the day's work** | |

**You should now know the principles of fluorescence and single point laser scanning microscopy and, critical tools/settings to acquire correct data/images**

| Tuesday 27 August | | |
|---|---|---|
| **09.00 – 11.15** | **Practical 1 (groups 4-6)** | |
| | Single point laser scanning microscopy | CFIM |
| **11.15** | **Coffee** | 15.2 |
| **11.30 – 12.30** | *Lecture* | |
| | Detectors and noise | THB |
| **12.30** | **Lunch** | |
| **13.15 – 14.20** | *Lecture*<br>Digital images – characteristics and measurements<br>Do's and don'ts, ethics in image acquisition and processing | THB |
| **14.30 – 15.30** | **Practical 2 (rotation 1)** | |
| | Dynamic range<br>Configuration of 3D stacks<br>Multichannel and time lapse<br>Spectral imaging | PHV<br>THB<br>THB<br>CP |
| **15.30** | **Coffee** | CFIM |
| **15.45 – 16.45** | **Practical 2 (rotations 2)** | |
| **16.45 – 17.00** | **Summary of the day's work** | |

**You should now know how to properly use detectors to acquire digital images (data) and how to acquire multi-dimensional data sets (x,y,z,t,λ...)**

| Wednesday 28 August | | |
|---|---|---|
| **09.00 – 10.00** | *Lecture* | |
| | *Live cell imaging* | THB |
| **10.00** | **Coffee** | 15.2 |
| **10.15 – 11.00** | *Lecture and demo* | |
| | *Deconvolution* | THB |
| **11.15 - 12.10** | *Lecture* | |
| | *Colocalization. from sample prep to analysis* | CP |
| **12.10 – 12.45** | **Lunch** | |
| **12.45 – 14.45** | **Practical 2 ( rotations 3 and 4)** | |
| **14.45** | **Coffee** | 15.2 |
| **15.00 – 15.45** | *Lecture* | |
| | *Intro to some F words* | CP |
| **15.45 – 16.15** | *Lecture* | |
| | **Linear super resolution** | PHV |
| | SIM, rescan confocal and Airy Scan | |
| | *Lecture* | |
| | Non-linear super resolution | |
| | STED and SMLM | |
| **16.45 – 17.00** | **Summary of the day's work** | |

**You have been introduced to several advanced bioimaging applications to investigate dynamic processes by cells or molecules, relative localization of molecules and high spatial resolution of intracellular structures**

| Thursday 29 August | | | |
|---|---|---|---|
| **09.00 – 09.40** | *Lecture*<br>FRAP - Fluorescence Recovery After Photobleaching | | DZ |
| **09.40** | **Coffee break** | | CFIM |
| **10.00 – 11.00** | ***Practical 3 - rotations 1*** | | CFIM |
| | ▪ Airy scan | JB | CPR |
| | ▪ FRAP | DZ | LSM780 |
| | ▪ Spinning disc | THB | CellObs |
| | ▪ Super Resolution (SIM) | PHV | Elyra PS.1 |
| **11.00** | **Break** | | CFIM |
| **11.15 – 12.15** | ***Practical 3 - rotations 2*** | | CFIM |
| **12.15** | **Lunch** | | |
| **13.00 – 14.00** | ***Practical 3 (rotation 3)*** | | CFIM |
| **14.00** | **Coffee** | | 15.2 |
| **14.15 – 15.15** | ***Practical 3 (rotation 4)*** | | CFIM |
| **15.15** | **Coffee** | | 15.2 |
| **15.30 – 16.30** | *Lecture*<br>FRET / FCCS | | 15.2 |
| **16.30- 17.00** | ***Summary of the day*** | | |
| **17.00 - ???** | **Evenning Lecture (TBD) and course dinner** | | **Faculty Club** |

**You should now understand the basics of FRAP, FRET, FCS, spinning disk, Airy Scan and SIM**

| Friday 30 August | | |
|---|---|---|
| **09.00 – 10.00** | *Practical 4 (rotation 1)* | CFIM |
| | ▪ FRET/FCS | DZ |
| | ▪ TIRF | THB |
| | ▪ Performance checks / linearity | CP |
| | ▪ SMLM | PHV |
| **10.00** | **Coffee** | CFIM |
| **10.15 – 11.10** | *Practical 4 - rotations 2* | CFIM |
| **11.15 – 12.15** | *Practical 4 - rotations 3* | |
| **12.15** | **Lunch** | |
| **13.00 – 14.00** | *Practical 4 - rotation 4* | CFIM |
| **14.00** | **Coffee** | 15.2 |
| **14.15 – 15.00** | *Lecture* | |
| | Fluorescence Localization After Photobleaching (FLAP) | DZ |
| **15.00** | **Break** | |
| **15.15 – 16.00** | *Lecture* | |
| | Choosing the right technique | CP |
| **16.00 – 16.30** | **Conclusions, Questions and Evaluation of the course** | |

**You should now know the essential to properly use fluorescence microscopy for your research**
**We hope you have also learned that generation of data with Microscopy should be taken seriously but……**

**MICROSCOPY IS FUN !!!!**