CFIM MICROSCOPY COURSE

PROGRAMME

PRINCIPLES OF MICROSCOPY

11.01.16-15.01.2016

CONFOCAL AND FLUORESCENCE MICROSCOPY

25.01.16-29.01.2016

PhD Course - University of Copenhagen
Department of Biomedical Sciences
Core Facility for Integrated Microscopy
in Collaboration with The Royal Microscopical Society

Monday 11 of January		
09:00 – 09:30	*Introduction*	KQ/CP
09:30 – 10:15	*Lecture* The story of the microscope	PJE/AS
10:15	**Coffee**	15.2
10:30 – 11:30	**Lecture** Limitations of the eye. Resolution, contrast, magnification. Lenses, magnifying glasses, compound microscopes.	PJE
11:30 – 11:45	**Break**	
11:45 – 12:45	**Lecture** Conjugate planes	
12:45	**Lunch**	
13:30 – 14:15	***Lecture*** Köhler illumination	PJE
14:15 – 15:00	***Practical 1 (rotation 1)***	
	Köhler illumination (4)	CP
	Conjugate planes on the optical bench (3)	AS
	Conjugate planes in the microscope (3)	PJE
	Workbook DIY (1 – 4)	THB/LP
15:00	**Coffee**	15.2
15:15 - 16:45	***Practical 1 (rotations 2 and 3)***	
16:45 – 17:00	**Summary of day's work; questions and workbook**	

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 12 of January	
09:00 – 09:45	*Practical 1 (rotation 4)*	
09:45	**Coffee**	**15.2**
10:00 – 11:00	*Lecture* Lens defects and their correction	
11:00 – 11:05	Short break	
11:05 – 11:30	*Demonstration* Setting up Köhler illumination in transmitted light Depth of field and depth of focus	PJE
11:30 – 12:15	*Lecture-demonstration* Diffraction, resolution and contrast	PJE
12:15	**Lunch**	
13:00 – 13:45	*Lecture-demonstration continued (video)*	PJE
13.45 – 14.30	*Practical 2 (rotation 1)* ▪ Diffraction experiments(6) ▪ Aperture (7) ▪ Resolving power (8) ▪ Work Book DIY (1-8)	PJE AS CP THB/LP
14:30	**Coffee**	**15.2**
14:45 – 15:30	*Practical 2 (rotation 2)*	
15:30 – 16:15	*Practical 2 (rotation 3)*	
16:15 – 17:00	*Summary of day's work; questions and workbook*	

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

Wednesday 13th of January		
09:00 – 09:45	***Practical*** *2 (rotation 4)*	
09:45	**Coffee**	**15.2**
10:00 – 11.00	***Lecture*** Contrast: Bright field, dark ground, Rheinberg, Phase contrast	PJE
11:00 – 12:00	***Practical*** *3* Dark field – patch stop (9) Rheinberg (10)	
12:00	**Lunch**	
12:45 – 13:45	***Lecture*** The nature and properties of light	AS
13:45	**Coffee**	**15.2**
14:00 – 15:00	Equations for limit of resolution of optical instruments	AS
15:00 – 16:30	***Practical 4*** Phase contrast (11)	
16.30 – 17.00	*Summary of day's work; questions and workbook*	
17.00 -	*Out for drinks with Andrew and Peter*	

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

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

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 15th of January		
9.00 – 9.15	*Lecture*	PJE
	Stereomicroscopes	
09.15 – 10.00	*Lecture*	PJE
	Principles of fluorescence and confocal microscope	
10.00	**Coffee**	**15.2**
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/LP
	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*	**15.2**
	Sample preparation – practical considerations	CP
15.15 – 15.45	**Questions; summary and evaluation of course**	

Now you know the principles; see you in a week.

Monday 25th of January			
9.00 – 09.30	*Lecture*	*CP*	*15.2.18*
	Intro to Fluorescence		
09.30 – 10.30	*Lecture*		
	Confocal Microscopy	LP	*15.2.18*
10.30	**Coffee**		15.2
10.45 – 12.15	*Lectures*		
	Confocal microscopy (cont)	LP	*15.2.18*
	Introduction to ZEN software	CP	
	Digital imaging	CP	
12.15	**Lunch**		
13.00 – 14.15	*Lecture- Remote session*		
	Digital imaging, imaging dimensions	CP	*15.2.18*
14.015	**Coffee**		15.2
14.30 – 16.30	**Practical 1 (groups 1-3)**		
	Single point laser scanning microscopy • Channel design • Bleed through/cross-excitation • Dynamic range/ SN ratio • Digital resolution		*CFIM*

Tuesday 26th of January			
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	*15.2.18*
12.30	**Lunch**		
13.15 – 14.30	*Lecture* Digital images – characteristics and measurements Do's and don'ts, ethics in image processing	THB	*15.2.18*
14.30 – 15.30	**Practical 2 (rotation 1)**		
	Dynamic range Configuration of 3D stacks Multichannel and time lapse Spectral imaging	LP THB THB CP	
15.30	**Coffee**		CFIM
15.45 – 16.45	**Practical 2 (rotations 2)**		

Wednesday 27th of January			
09.00 – 10.00	*Lecture*		*15.2.18*
	Live cell imaging	THB	
10.00	**Coffee**		*15.2*
10.15 – 11.00	*Lecture and demo*		*15.2.18*
	Deconvolution	THB	
11.00 - 11.45	*Lecture*		
	Colocalization: from sample prep to analysis	CP	
11.45	**Lunch**		
12.30 – 14.30	Practical 2 (rotations 3 and 4)		*CFIM*
14.30	**Coffee**		
14.45 – 15.45	*Lecture*		
	Super resolution (SIM, STED, localization microscopy)	LP	*15.2.18*
15.45 – 16.30	*Lecture*		
	Intro to some F words	CP	*15.2.18*

Thursday 28th January			
09.00 – 09.40	*Lecture* FRAP - Fluorescence Recovery After Photobleaching	*DZ*	*15.2.18*
09.40	**Coffee**		CFIM
10.00 – 12.15	***Practical 3 (rotations 1 and 2)***		*CFIM*
	▪ Own sample	CP	*LSM710*
	▪ FRAP	DZ	*LSM780*
	▪ Spinning disc	THB	*CellObs*
	▪ Super Resolution (SIM)	LP	*Elyra PS.1*
12.15	**Lunch**		
13.00 – 14.00	***Practical 3 (rotation 3)***		
14.00	**Coffee**		*15.2*
14.15 – 15.15	***Practical 3 (rotation 4)***		
15.15	**Coffee**		
15.30 – 16.30	*Lecture* FRET / FCCS		
17.30- ?	***Evening lecture "Light sheet microscopy for multiview imaging of large specimens" by Maria Trulsson, Zeiss and course dinner sponsored by ZEISS***		*Faculty club 16.6.16*

Friday 29th January			
09.00 – 10.00	*Practical 4 (rotation 1)*		*CFIM*
	▪ FRET/FCS		DZ
	▪ TIRF		THB
	▪ Performance checks / linearity		CP
	▪ DIC / Tiles / Positions		LP
10.00	**Coffee**		CFIM
10.15 – 12.15	*Practical 4 (rotations 2 and 3)*		*CFIM*
12.15	**Lunch**		
13.00 – 14.00	*Practical 4 (rotation 4)*		*CFIM*
14.00	**Coffee**		*CFIM*
14.15 – 15.00	*Lecture*		
	Fluorescence Localization After Photobleaching (FLAP)	DZ	*15.2.18*
15.00	**Coffee**		
15.15 – 16.00	*Choosing the right technique*	LP	*15.2.18*
16.00	**Conclusions, Questions and Evaluation of the week**		