CFIM MICROSCOPY COURSE

PROGRAMME

PRINCIPLES OF MICROSCOPY

MONDAY 12TH OF JANUARY 2015 – FRIDAY 16TH OF JANUARY 2015

CONFOCAL AND FLUORESCENCE MICROSCOPY

MONDAY 26TH OF JANUARY 2015 – FRIDAY 30TH OF JANUARY 2015

PhD Course - University of Copenhagen

Department of Biomedical Sciences

Core Facility for Integrated Microscopy

in Collaboration with The Royal Microscopical Society

Monday 12th 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, 9, and 10)	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 13th of January		
09:00 – 09:45	*Practical 1 (rotation 4)*	
09:45	**Coffee**	**15.2**
10:00 – 10:45	*Lecture* Lens defects and their correction	
10:45 – 11:30	*Demonstration* Setting up Köhler illumination in transmitted light Depth of field and depth of focus	PJE
11:30 – 12:30	*Lecture-demonstration* Diffraction, resolution and contrast	PJE
12:30	**Lunch**	
13:15 – 14:00	*Lecture-demonstration continued*	PJE
14.00 – 14.45	*Practical 2 (rotation 1)* ▪ Diffraction experiments ▪ Aperture (7) ▪ Resolving power (9,12, and 13) ▪ Work Book DIY (continue + 4, 6 - 9)	PJE AS CP THB/LP
14:45	**Coffee**	**15.2**
15:00 – 15:45	*Practical 2 (rotation 2)*	
15:45 – 16:30	*Practical 2 (rotation 3)*	
16:30 – 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 14th of January		
09:00 – 09:45	***Practical** 2 (rotation 4)*	
09:45	**Coffee**	**15.2**
10:00 – 11.00	***Lecture***	PJE
	Contrast: Bright field, dark ground, Rheinberg,	
	Phase contrast	
11:00 – 12:00	***Practical** 3*	
	Dark field – patch stop (13)	
	Rheinberg (14)	
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 (15)	
16.30 – 17.00	*Summary of day's work; questions and workbook*	
17.00 -	*Course dinner - Lecture from the Nobel prize in Chemistry 2014 for their work in developing Super resolution microscopy solutions: Erik Betzig, Stefan W Hell and William E Moerner.*	

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

Thursday 15th 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 (17) Effects of mounting media	
11.15 – 11.45	***Lecture*** *Understanding interference colours*	AS
11.45	**Lunch**	
12.30 – 13.15	***Lecture*** Differential interference contrast	PJE
13.15 – 14.15	*Practical 6 (rotation 1 and 2)* ▪ Polarised light: examples at lightbox (16) ▪ DIC (Epi-illumination and transmitted light) (18) ▪ DIC on a Laser Scanning Microscope ▪ Workbook (continue + 19)	 AS PJE CP THB/LP
14.15	**Coffee**	**15.2**
14.30 – 15.30	*Practical 6 (rotation 3 and 4)*	
15.30 – 16.45	*Lecture* Principles of fluorescence and confocal microscope	 PJE
16.45 – 17.00	*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 16th of January		
09.00 – 09.30	*Lecture* Methods of recording images	PJE
09.30 – 10.30	*Lecture* Principles of digital image recording Optical considerations in fitting a camera to a microscope	PJE
10.30	**Coffee**	**15.2**
10.45 – 11.30	*Lecture* Stereomicroscopes	PJE
11.30 – 12.00	*Lecture* Measurement, cleaning and maintenance	PJE
12.00	**Lunch**	
12.45 – 14.15	*Lecture* Principles of electron microscopy	PJE /AS
14.10	**Coffee**	**15.2**
14.30 – 16.30	*Practical 7* ▪ Transmission electron microscopy ▪ Scanning electron microscopy ▪ Alignment of the Hg arc ▪ Fluorescence	 RL KQ CP THB/LP
16.30 – 17.00	*Questions; summary of course*	

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

Monday 26th of January			
9.00 – 09.15	*Welcome & introduction*	*KQ*	*15.2.18*
09.15 – 10.15	*Lecture*		
	Atoms, light and matter	AE	*15.2.18*
10.15	**Coffee**		**15.2**
10.30 – 11.30	*Lecture*		
	Fluorescence and fluorophores	AE	*15.2.18*
11.30 – 12.45	*Interactive Lecture*		
	Computers and software	AE	*15.2.18*
13.00	**Lunch**		
13.45 – 14.45	*Lecture*		
	Fluorescence microscopy: an overview.	AE	*15.2.18*
14.45 – 15.15	*Interactive lecture*	JC	*15.2.18*
	3D Reconstruction		
15.15	**Coffee**		**15.2**
15.30 – 16.40	*Interactive lecture*		
	3D Reconstruction	JC	*15.2.18*
16.40 – 17.00	*Lecture*		
	Fluorescence microscopy: an overview (cont.)	AE	*15.2.18*

Tuesday 27th of January			
09.00 – 10.00	*Lecture*		
	Signals, noise and detectors	AE	*15.2.18*
10.00	**Coffee**		15.2
10.15 – 11.15	*Lecture*		
	Confocal and wide-field fluorescence microscopy	AE	*15.2.18*
11.15 – 11.30	**Coffee**		**15.2**
11.30 – 12.30	*Lecture*		
	Photon sensing arrays	AE	*15.2.18*
12.30	**Lunch**		
13.15 – 14.15	*Lecture*		
	Confocal and wide-field fluorescence microscopy (cont.)	AE	*15.2.18*
14.15	**Coffee**		CFIM
14.30 – 16.00	*Practical 1 in 5 groups (rotations 1 and 2)*		
			CFIM
	▪ Configuring a confocal microscope	LP	*LSM710*
	▪ Collecting 3D data and sampling	JC	*LSM700*
	▪ Collecting spectral data	CP	*LSM780*
	▪ CCD and CMOS cameras	AE	*Elyra PS.1*
			18.1
	▪ Cross-talk and collecting confocal images	THB	*LSM710*
16.00	**Coffee**		CFIM
16.15 – 17.00	*Practical 1 continued (rotation 3)*		*CFIM*

Wednesday 28th of January			
09.00 – 10.30	*Practical 1 continued (rotations 4 and 5)*		*CFIM*
10.30	**Coffee**		*15.2*
10.45 – 11.45	*Interactive lecture*		
	Deconvolution and image restoration	JC	*15.2.18*
11.45	**Coffee**		15.2
12.00 – 13.00	*Interactive lecture*		
	Deconvolution and image restoration (cont.)	JC	*15.2.18*
13.00	**Lunch**		
13.45 – 14.45	*Lecture*		
	Quantification of Fluorescence	AE	*15.2.18*
14.45	**Coffee**		15.2
15.00 – 16.00	*Lecture*		
	Beyond the diffraction limit	JC	*15.2.18*
16.00 – 17.00	*Lecture*		
	Immunofluorescence and affinity fluorescent staining	AE	*15.2.18*

Thursday 29th of January			
09.00 – 09.45	*Lecture*		
	FRAP - Fluorescence Recovery After Photobleaching	*DZ*	*15.2.18*
09.45	**Coffee**		15.2
10.00 – 10.55	*Lecture*		
	FRET - Fluorescent Resonance Energy Transfer and	DZ	*15.2.18*
	FCCS - Fluorescence Cross-Correlation Spectroscopy		

Thursday 29th of January			
11.00 – 13.00	*Practical 2 (rotation 1)*		*CFIM*
	▪ Checking the confocal microscope	AE	*LSM710*
	▪ 3D reconstruction	JC	*21.01.24A*
	▪ FRAP, FRET & FCS	DZ	*LSM780*
	▪ Spinning disc	THB	*CellObs*
	▪ Super Resolution	LP	*Elyra PS.1*
13.00	**Lunch**		
13.45 – 15.45	*Practical 2 continued (rotation 2)*		
15.45	**Coffee**		*15.2*
16.00 – 17.00	*Lecture*		
	Creating micrographs from digital data	AE	*15.2.18*
17.00 -	***Social Event***		

Friday 30th of January			
09.00 – 11.00	*Practical 2 continued– (rotation 3)*		*CFIM*
11.00	**Coffee**		CFIM
11.15 – 13.15	*Practical 2 continued– (rotation 4)*		*CFIM*
13.15	**Lunch**		
14.00 – 16.00	*Practical 2 continued– (rotation 5)*		
16.00	**Coffee**		*CFIM*
16.15 – 17.00	*Lecture*		
	Fluorescence Localization After Photobleaching (FLAP)	DZ	*15.2.18*