

CFIM MICROSCOPY COURSE

PROGRAMME

PRINCIPLES OF MICROSCOPY – INTRO COURSE

FRIDAY 16TH OF AUGUST

CONFOCAL AND FLUORESCENCE MICROSCOPY

MONDAY 26TH – FRIDAY 30TH OF AUGUST 2013

PRINCIPLES OF MICROSCOPY

MONDAY 13TH – FRIDAY 17TH OF JANUARY 2014

PHD COURSE

UNIVERSITY OF COPENHAGEN

AUGUST 2013 / JANUARY 2014

DEPARTMENT OF BIOMEDICAL SCIENCES

IN COLLABORATION WITH

THE ROYAL MICROSCOPICAL SOCIETY



Friday 16th of August

09.00 – 10.00	<i>Lecture</i>	LP / CP	15.2.18
	Properties of light. Resolution and magnification		
	The microscope		
10.00 - 10.15	Coffee		
10.15 – 11.15	<i>Lecture</i>	CP/THB	15.2.18
	Conjugate planes		
	Fluorescence		
11:15 – 11:30	Coffee		
11:30 – 12:30	Practical		
	▪ Conjugate planes on the optical bench		KQ
	▪ Aperture		LP
	▪ Fluorescence		THB
	▪ Resolution		CP
12.30 – 13.15	Lunch		
13.15 – 14.15	Practical (cont.)		
14.15 – 15.15	<i>Interactive lecture</i>		15.2.18
	Köhler illumination		CP/LP/THB/KQ

Important concepts to understand and remember: Resolution, magnification, conjugate planes, Köhler illumination and fluorescence. See you in a week.

Monday 26th of August

9.00 – 09.15	<i>Welcome & introduction</i>	KQ	15.2.18
09.15 – 10.15	<i>Lecture</i>		15.2.18
	Atoms, light and matter	AE	
10.15	Coffee		
10.30 – 11.30	<i>Lecture</i>		15.2.18
	Fluorescence and fluorophores	AE	
11.30 – 12.45	<i>Interactive Lecture</i>		15.2.18
	Computers and software	AE	
13.00	Lunch		
13.45 – 14.45	<i>Lecture</i>		15.2.18
	Fluorescence microscopy: an overview.	AE	
14.45 – 15.15	<i>Interactive lecture</i>		15.2.18
	Fluorescence microscopy: the stand		
15.15	Coffee		
15.30 – 16.40	<i>Lecture</i>		15.2.18
	Signal, noise and detectors	AE	
16.40 – 17.00	<i>Lecture</i>		15.2.18
	Fluorescence microscopy: an overview (cont.)	AE	

Tuesday 27th of August

09.00 – 10.00	<i>Lecture</i>		<i>15.2.18</i>
	Confocal and wide-field fluorescence microscopy	AE	
10.00	Coffee		<i>15.2.18</i>
10.15 – 11.15	<i>Lecture</i>		<i>15.2.18</i>
	Photon sensing arrays	LH	
11.15 – 12.15	<i>Lecture continued</i>		<i>15.2.18</i>
	Confocal and wide-field fluorescence microscopy	AE	
12:15 – 13:00	<i>Practical in 5 groups – 1 rotation</i>		<i>CFIM</i>
	<ul style="list-style-type: none"> ▪ Zeiss LSM 710 Configuring a confocal microscope ▪ Zeiss LSM700 Collecting 3D data and sampling ▪ Zeiss LSM 780 Collecting spectral data ▪ Zeiss cell observer TIRF microscopy ▪ Digital cameras 	AE JC LP/CP TH LH	
13.00	Lunch		
13.45 – 15.15	<i>Practical continued – 2 rotations</i>		<i>CFIM</i>
15.15	Coffee		<i>CFIM</i>
15.30 – 17.00	<i>Practical continued – 2 rotations</i>		<i>CFIM</i>

Wednesday 28th of August

09.00 – 10.00	<i>Lecture</i>		<i>15.2.18</i>
	3D Reconstruction	JC	
10.00	Coffee		<i>15.2.18</i>
10.15 – 11.15	<i>Lecture continued</i>		<i>15.2.18</i>
	3D Reconstruction c	JC	
11.15 – 12.15	<i>Lecture</i>		<i>15.2.18</i>
	Quantification of Fluorescence	AE	
12:15 – 13:00	<i>Interactive lecture</i>		<i>15.2.18</i>
	Deconvolution and Image restoration	JC	
13.00	Lunch		
13.45 – 14.45	<i>Interactive lecture continued</i>		<i>15.2.18</i>
	Deconvolution and Image restoration	JC	
14.45 – 15.45	<i>Lecture</i>		
	Immunofluorescence and affinity fluorescent staining	AE	
15.45	Coffee		<i>15.2.18</i>
15.30 – 17.00	<i>Lecture</i>		<i>15.2.18</i>
	Beyond the diffraction limit	JC	

Thursday 29th of August

09.00 – 09.45	<i>Lecture</i> Fluorescence Recovery After Photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS)	DZ	15.2.18
09.45	Coffee		15.2.18
10.00 – 11.00	<i>Lecture</i> Fluorescent Resonance Energy Transfer (FRET)c	DZ	15.2.18
11.00 – 13.00	<i>Practical – 1 rotation</i> <ul style="list-style-type: none"> ▪ Zeiss LSM 710 Checking the confocal microscope ▪ 3D reconstruction ▪ Zeiss LSM 780 FRAP, FRET & FCS ▪ TIRF, Spinning disc ▪ LSM 700 collecting confocal data (1h 15 min) & Fluorescence, alignment of the Hg arc (45 min) 	 AE JC DZ THB LP/CP KQ	 <i>CFIM</i> <i>CFIM</i> <i>CFIM</i> <i>CFIM</i> <i>CFIM</i> 15.2.10
13.00	Lunch		
13.45 – 15.45	<i>Practical continued– 1 rotation</i>		
15.45	Coffee		15.2.18
16.00 – 17.00	<i>Lecture</i> Creating micrographs from digital data	AE	15.2.18

Friday 30th of August

09.00 – 11.00	<i>Practical continued– 1 rotation</i>	<i>CFIM</i>
11.00	Coffee	<i>CFIM</i>
11.15 – 13.15	<i>Practical continued– 1 rotation</i>	<i>CFIM</i>
13.15	Lunch	
14.00 – 16.00	<i>Practical continued– 1 rotation</i>	
16.00	Coffee	<i>CFIM</i>
16.15 – 17.00	<i>Lecture</i>	<i>15.2.18</i>
	Fluorescence Localization After Photobleaching (FLAP)	DZ

Monday 13th of January

09:00 – 09:30	<i>Introduction</i>	KQ
09:30 – 10:15	<i>Lecture</i> The story of the microscope	PJE/AS
10:15	Coffee	
10:30 – 12:45	<i>Lecture</i> Limitations of the eye. Resolution, contrast, magnification. Lenses, magnifying glasses, compound microscopes. Conjugate planes	PJE
12:45	Lunch	
13:30 – 15:00	<i>Lecture</i> Lens defects and their correction Köhler illumination	PJE
15:00	Coffee	
15:15 – 16:45	<i>Practical 1</i> <ul style="list-style-type: none"> ▪ Köhler illumination (4) ▪ Conjugate planes on the optical bench (3) ▪ Conjugate planes in the microscope (3) ▪ Workbook DIY (1 – 4, 9, and 10) 	KQ AS PJE THB/CP/LP
16:45 – 17:00	<i>Summary of day's work; questions and workbook</i>	

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 14th of January

09:00 – 09:45	<i>Practical 1 continued</i>	
09:45	Coffee	
10:00 – 10:45	<i>Practical 1 continued</i>	
10:45 – 11:30	<i>Demonstration</i> Setting up Köhler illumination in transmitted light Depth of field and depth of focus	
11:30 – 12:30	<i>Lecture-demonstration</i> Diffraction, resolution and contrast	PJE
12:30	Lunch	
13:15 – 14:00	<i>Lecture-demonstration continued</i>	PJE
14.00 – 14.45	<i>Practical 2</i> <ul style="list-style-type: none"> ▪ Diffraction experiments ▪ Aperture (7) ▪ Resolving power (9,12, and 13) ▪ Work Book DIY (continue + 4, 6 - 9) 	KQ AS PJE THB/CP/LP
14:45	Coffee	
15:00 – 15:45	<i>Practical 2 continued</i>	
15:45 – 16:45	<i>Lecture</i> Equations for limit of resolution of optical instruments	AS
16:45 – 17:00	<i>Summary of day's work; questions and workbook</i>	

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

Wednesday 15th of January

09:00 – 09:45	<i>Practical 2 continued</i>	
09:45	Coffee	
10:00 – 10:45	<i>Practical 2 continued</i>	
10:45 – 11:45	<i>Lecture</i> Contrast: Bright field, dark ground, Rheinberg, Phase contrast	PJE
11:45	Lunch	
12:30 – 14:30	<i>Practical 3</i> Dark field – patch stop (13) Rheinberg (14)	
14:30	Coffee	
14:45 – 15:45	<i>Lecture</i> The nature and properties of light	AS
15.45 – 16.15	<i>Summary of day's work; questions and workbook</i>	

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

Thursday 16th of January

09.00 – 10.00	<i>Practical 4</i> Phase contrast (15)	
10.00	Coffee	
10.15 – 11.15	<i>Lecture-demonstration</i> Polarised light	AS
11.15 – 12.30	<i>Practical 5</i> <ul style="list-style-type: none"> ▪ Contrast in the polarised-light microscope (17) ▪ Effects of mounting media 	
12.30	Lunch	
13.15 – 13.45	<i>Lecture</i> Understanding interference colours	AS
13.45 – 14.30	<i>Lecture</i> Differential interference contrast	PJE
14.30	Coffee	
14.45 – 16.15	<i>Practical 6</i> <ul style="list-style-type: none"> ▪ Polarised light: examples at lightbox (16) ▪ DIC (Epi-illumination and transmitted light) (18) ▪ CFIM introduction ▪ Workbook (continue + 19) 	AS PJE KQ THB/CP/LP
16.15 – 16.45	<i>Lecture</i> Principles of the confocal microscope	PJE
16.45 – 17.00	<i>Summary of day's work; questions and workbook</i>	
18.00 -	Social event	

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 17th of January

09.00 – 09.30	<i>Lecture</i> Methods of recording images	PJE
09.30 – 10.30	<i>Lecture</i> Principles of digital image recording Optical considerations in fitting a camera to a microscope	PJE
10.30	Coffee	
10.45 – 11.30	<i>Lecture</i> Stereomicroscopes	PJE
11.30 – 12.00	<i>Lecture</i> Cleaning and maintenance	PJE
12.00 – 12.45	Lunch	
12.45 – 14.15	<i>Lecture</i> Principles of electron microscopy	PJE /AS
14.10 – 14.30	Coffee	
14.30 – 16.30	<i>Practical 7</i> <ul style="list-style-type: none"> ▪ Transmission electron microscopy ▪ Scanning electron microscopy ▪ Image recording; fitting the camera (20) ▪ Fluorescence 	RL KQ PJE THB/CP/LP
16.30 – 17.00	<i>Questions; summary of course</i>	