

# CFIM MICROSCOPY COURSE

## TIMETABLE

### **PRINCIPLES OF MICROSCOPY**

MONDAY 6<sup>TH</sup> OF JANUARY 2014 – FRIDAY 10<sup>TH</sup> OF JANUARY 2014

### **CONFOCAL AND FLUORESCENCE MICROSCOPY**

MONDAY 20<sup>TH</sup> OF JANUARY 2014 – FRIDAY 24<sup>TH</sup> OF JANUARY 2014

PHD COURSE

UNIVERSITY OF COPENHAGEN

JANUARY 2014

DEPARTMENT OF BIOMEDICAL SCIENCES

IN COLLABORATION WITH

THE ROYAL MICROSCOPICAL SOCIETY



## Monday 6<sup>th</sup> 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	<b>Coffee</b>	
10:30 – 12:45	<i>Lecture</i> Limitations of the eye. Resolution, contrast, magnification. Lenses, magnifying glasses, compound microscopes. Conjugate planes	PJE
12:45	<b>Lunch</b>	
13:30 – 15:00	<i>Lecture</i> Lens defects and their correction Köhler illumination	PJE
15:00	<b>Coffee</b>	
15:15 – 16:45	<i>Practical 1</i> <ul style="list-style-type: none"><li>▪ Köhler illumination (4)</li><li>▪ Conjugate planes on the optical bench (3)</li><li>▪ Conjugate planes in the microscope (3)</li><li>▪ Workbook DIY (1 – 4, 9, and 10)</li></ul>	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 7<sup>th</sup> of January

<b>09:00 – 09:45</b>	<i>Practical 1 continued</i>	
<b>09:45</b>	<b>Coffee</b>	
<b>10:00 – 10:45</b>	<i>Practical 1 continued</i>	
<b>10:45 – 11:30</b>	<i>Demonstration</i> Setting up Köhler illumination in transmitted light Depth of field and depth of focus	
<b>11:30 – 12:30</b>	<i>Lecture-demonstration</i> Diffraction, resolution and contrast	PJE
<b>12:30</b>	<b>Lunch</b>	
<b>13:15 – 14:00</b>	<i>Lecture-demonstration continued</i>	PJE
<b>14.00 – 14.45</b>	<i>Practical 2</i> <ul style="list-style-type: none"><li>▪ Diffraction experiments</li><li>▪ Aperture (7)</li><li>▪ Resolving power (9,12, and 13)</li><li>▪ Work Book DIY (continue + 4, 6 - 9)</li></ul>	KQ AS PJE THB/CP/LP
<b>14:45</b>	<b>Coffee</b>	
<b>15:00 – 15:45</b>	<i>Practical 2 continued</i>	
<b>15:45 – 16:45</b>	<i>Lecture</i> Equations for limit of resolution of optical instruments	AS
<b>16:45 – 17:00</b>	<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 8<sup>th</sup> of January

<b>09:00 – 09:45</b>	<i>Practical 2 continued</i>	
<b>09:45</b>	<b>Coffee</b>	
<b>10:00 – 10:45</b>	<i>Practical 2 continued</i>	
<b>10:45 – 11:45</b>	<i>Lecture</i> Contrast: Bright field, dark ground, Rheinberg, Phase contrast	PJE
<b>11:45</b>	<b>Lunch</b>	
<b>12:30 – 14:30</b>	<i>Practical 3</i> Dark field – patch stop (13) Rheinberg (14)	
<b>14:30</b>	<b>Coffee</b>	
<b>14:45 – 15:45</b>	<i>Lecture</i> The nature and properties of light	AS
<b>15.45 – 16.15</b>	<i>Summary of day's work; questions and workbook</i>	
<b>17.00 -</b>	<i>Dinner and Invited lecture at the Faculty Club</i>	

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

## Thursday 9<sup>th</sup> of January

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

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 10<sup>th</sup> of January

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

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

**Monday 20<sup>th</sup> of January**

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

**Tuesday 21<sup>th</sup> of January**

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



## Wednesday 22<sup>th</sup> of January

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

### Thursday 23<sup>th</sup> of January

<b>09.00 – 09.45</b>	<i>Lecture</i>	DZ	15.2.18
	Fluorescence Recovery After Photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS)		
<b>09.45</b>	<b>Coffee</b>		15.2.18
<b>10.00 – 11.00</b>	<i>Lecture</i>		15.2.18
	Fluorescent Resonance Energy Transfer (FRET)c	DZ	
<b>11.00 – 13.00</b>	<i>Practical – 1 rotation</i>		
	▪ Zeiss LSM 710 Checking the confocal microscope	AE	CFIM
	▪ 3D reconstruction	JC	CFIM
	▪ Zeiss LSM 780 FRAP, FRET & FCS	DZ	CFIM
	▪ TIRF, Spinning disc	THB	CFIM
	▪ LSM 700 collecting confocal data (1h 15 min) & Fluorescence, alignment of the Hg arc (45 min)	CP KQ	CFIM 15.2.10
<b>13.00</b>	<b>Lunch</b>		
<b>13.45 – 15.45</b>	<i>Practical continued– 1 rotation</i>		
<b>15.45</b>	<b>Coffee</b>		15.2.18
<b>16.00 – 17.00</b>	<i>Lecture</i>		15.2.18
	Creating micrographs from digital data	AE	

### Friday 24<sup>th</sup> of January

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