CFIM Microscopy Course - Principles of Microscopy

Monday 6 August 2012 – Friday 10th of August

Principles of Microscopy - Day 1		
09:00 – 09:30	Introduction	Klaus Qvortrup
09:30 – 10:15	*Lecture* The story of the microscope	Peter Evennett/ Chris Hammond
10:15	Coffee	
10:30 – 12:45	*Lecture* Limitations of the eye. Resolution, contrast, magnification. Lenses, magnifying glasses, compound microscopes. Conjugate planes	Peter Evennett
12:45	Lunch	
13:30 – 15:00	*Lecture* Lens defects and their correction Köhler illumination	Peter Evennett
15:00	Coffee	
15:15 – 16:30	*Practicals* ▪ Köhler illumination ▪ Conjugate planes on the optical bench ▪ Conjugate planes in the microscope ▪ Workbook DIY (1 – 5, 10, 11, and 14)	 Klaus Qvortrup Chris Hammond Peter Evennett Thomas Braunstein
16:30 – 16:45	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.		

Principles of Microscopy - Day 2		
09:00 – 10:15	*Practicals continued*	
10:15	Coffee	
10:30 – 11:15	*Demonstration* Setting up Köhler illumination in transmitted light Depth of field and depth of focus	
11:15 – 13:00	*Lecture-demonstration* Diffraction, resolution and contrast	Peter Evennett
13:00	Lunch	
13.45 – 15.45	*Practicals* ▪ Diffraction experiments ▪ Aperture (p. 15) ▪ Resolving power (p. 17) ▪ Work Book DIY (p. 4, 7 - 9)	 Peter Evennett Chris Hammond Klaus Qvortrup Thomas Braunstein
15:45	Coffee	
16:00 – 16:45	*Practicals continued*	
16:45 – 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.		

Principles of Microscopy - Day 3		
09:00 – 09:45	*Lecture* Equations for limit of resolution of optical instruments	Chris Hammond
09:45	Coffee	
10:00 – 11:00	*Lecture* Contrast: Bright field, dark ground, Rheinberg, Phase contrast	Peter Evennett
11:15 – 12:00	*Practicals* ▪ Dark field – patch stop (p. 26) ▪ Rheinberg	 Peter Evennett Chris Hammond
12:00 – 13:00	Lunch	
13:00 – 14:30	*Practicals (continued)*	
14:30 – 15:00	Coffee *(exchange microscopes)*	
15:00 – 16:30	Practicals Phase contrast (p. 28)	Peter Evennett Chris Hammond Klaus Qvortrup
You should now understand how the properties of specimens may be exploited in the microscope to give rise to contrast.		

Principles of Microscopy - Day 4		
09.00 – 09.45	*Lecture* The nature and properties of light	Chris Hammond
09.45 – 10.00	Coffee	
10.00 – 11.00	*Lecture-demonstration* Polarised light	Chris Hammond
11.00 – 11.30	*Practical* ▪ Contrast in the polarised-light microscope ▪ Effects of mounting media	
1130 – 1145	Coffee	
1145 – 1230	*Practicals continued*	
1230 – 1300	*Lecture* Understanding interference colours	Chris Hammond
1300 - 1345	Lunch	
13.45 – 14.30	*Lecture* Differential interference contrast	Peter Evennett
14.30 - 1445	Coffee	
14.45 – 16.45	*Practicals* ▪ Polarised light: examples at lightbox ▪ DIC (Epi-illumination and transmitted light) ▪ CFIM introduction ▪ Workbook (17 - 19)	 Chris Hammond Peter Evennett Klaus qvortrup Thomas Braunstein
16.15 – 16.45	*Lecture* Principles of the confocal microscope	Peter Evennett
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.		

Principles of Microscopy - Day 5		
09.00 – 09.30	*Lecture* Methods of recording images	Peter Evennett
09.30 – 10.30	*Lecture* Principles of digital image recording Optical considerations in fitting a camera to a microscope	Peter Evennett
10.30 – 10.45	Coffee	
10.45 – 11.30	*Lecture* Stereomicroscopes	Peter Evennett
11.30 – 12.00	*Lecture* Cleaning and maintenance	Peter Evennett
12.00 – 12.45	Lunch	
12.45 – 14.15	*Lecture* Principles of electron microscopy	Peter Evennett/ Chris Hammond
14.10 – 14.30	Coffee	
14.30 – 16.30	*Practical* ▪ Transmission electron microscopy ▪ Scanning electron microscopy ▪ Image recording; fitting the camera ▪ Methods of stereoscopic viewing	 Ramon Llebrechts Klaus Qvortrup Peter Evennett Chris Hammond
Now you know the principles; see you in a week.		

CFIM Microscopy Course
Confocal and Fluorescence Microscopy

Monday 13th of August – Friday 17th of August

Confocal and Fluorescence Microscopy - Day 1		
09.00 – 09.15	Welcome & introduction	Klaus Qvortrup
09.15 – 10.30	*Lecture* Atoms, light and matter	Alan Entwistle
10.30	Coffee	
10.45 – 11.45	*Lecture* Fluorescence and fluorophores	Alan Entwistle
11.45 – 13.00	*Interactive lecture* Computers and software	John Cookson/ Alan Entwistle
13.00	Lunch	
13.45 – 14.45	*Lecture* Fluorescence microscopy: an overview.	Alan Entwistle
14.45 – 15.15	*Interactive* lecture Fluorescence microscopy: the stand	Alan Entwistle
15.15	Coffee	
15.30 – 16.40	*Lecture* Signals, noise and detectors. Signal, noise and detectors	Alan Entwistle
16.40 – 17.00	*Lecture* Fluorescence microscopy: an overview (cont.)	Alan Entwistle

Confocal and Fluorescence Microscopy - Day 2		
09.00 – 10.00	*Lecture* Confocal and wide-field fluorescence microscopy	Alan Entwistle
10.00	Coffee	
10.15 – 11.15	*Lecture* CCD cameras and detecting fluorescence	Alan Entwistle
11.15 – 12.15	*Lecture* Confocal and wide-field fluorescence microscopy (cont.)	Alan Entwistle
12.15 – 17.00	*Practical in 5 groups* ▪ Zeiss LSM 710 Integration time and pixel density ▪ Zeiss LSM 700 Collect 3D data, discuss sampling ▪ Zeiss LSM 780 Spectral collection ▪ Zeiss Cell observer TIRF SD Intro live cell ▪ Digital cameras, Andor	 Alan Entwistle John Cookson Laure Plantard Thomas Braunstein Jørn Breumlund

Confocal and Fluorescence Microscopy - Day 3		
09.00 – 10.00	*Lecture* 3D Reconstruction	John Cookson
10.00	Coffee	
10.15 – 11.15	*Lecture* 3D Reconstruction	John Cookson
11.15 – 12.15	*Lecture* Quantification of fluorescence.	Alan Entwistle
12.15 – 13.00	*Interactive* lecture Deconvolution and image restoration	John Cookson
13.00	Lunch	
13.45 – 14.45	*Interactive* lecture Deconvolution and image restoration (cont.)	John Cookson
14.45 – 15.45	*Lecture* Immunofluorescence and affinity fluorescent staining	Alan Entwistle
15.45	Coffee	
16.00 – 17.00	*Lecture* Beyond the diffraction limit	John Cookson
Confocal and Fluorescence Microscopy - Day 4		
09.00 - 09.45	*Lecture* Fluorescence Recovery After Photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS)	Daniel Zicha
09.45	Coffee	
10.00 – 11.00	*Lecture* Fluorescent Resonance Energy Transfer (FRET)	Daniel Zicha
11.00 – 13.00	*Practicals* ▪ Zeiss LSM 710 Checking the confocal microscope ▪ 3D reconstruction ▪ Zeiss LSM 780 FRAP, FRET & FCS ▪ TIRF, Spinning disc ▪ Zeiss LSM 700 collecting confocal data (1h) Fluorescence, alignment of the Hg arc (1 h)	 Alan Entwistle John Cookson Daniel Zicha Thomas Braunstein Laure Plantard Klaus Qvortrup
13.00	Lunch	
13.45 – 15.45	*Practicals (continued)*	
15.45	Coffee	
16.00 – 17.00	*Lecture* Creating micrographs from digital data	 Alan Entwistle
18.00	Social Event	

Confocal and Fluorescence Microscopy - Day 5		
9.00 – 11.00	*Practicals (continued)* ▪ Zeiss LSM 710 Checking the confocal microscope ▪ 3D reconstruction ▪ Zeiss LSM 780 FRAP, FRET & FCS ▪ TIRF, Spinning disc ▪ Zeiss LSM 700 collecting confocal data (1h) Fluorescence, alignment of the Hg arc (1 h)	 Alan Entwistle John Cookson Daniel Zicha Thomas Braunstein Laure Plantard Klaus Qvortrup
11.00	Coffee	
11.15 – 13.15	*Practicals (continued)*	
13.15	Lunch	
14.00 – 16.00	*Practicals (continued)*	
16.00	Coffee	
16.15 – 17.00	*Lecture* Fluorescence Localization After Photobleaching (FLAP)	Daniel Zicha