

Advanced Imaging Techniques in Microscopy

An intense 5-day workshop providing a theoretical and practical overview of advanced microscopy and image analysis techniques for life science students, *Ph.D.* students, postdocs and technicians.

April 16th – 20th 2018

Life Imaging Center (LIC), Center for Biological Systems Analysis (ZBSA)
Albert-Ludwigs University Freiburg
Habsburgerstr. 49, 79104 Freiburg im Breisgau

Organization: Microscopy and Image Analysis Platform (MIAP) & Life Imaging Center (LIC)
Instructors: Roland Nitschke, Iris Bierschenk, Elitsa Bodurova, Sabine Haxelmans, Angela Naumann, Tobias Wernet, Martin Zeeb

Day 1: April 16th, 08:30 – 18:00	
08:30 – 09:15	Introduction: General workshop introduction; Overview of imaging methods
09:15 – 10:30	Lecture: Introduction to microscopy: History of microscopy, properties of light, microscope types, objectives, parfocality, refractive index, resolution, contrast methods, illumination and alignment
10:30 – 10:45	Break: Coffee and Tea
10:45 – 12:00	Lecture: Immunofluorescence labelling, sample preparation, cover glasses, mounting media and common pitfalls
12:00 – 13:00	LUNCH BREAK
13:00 – 14:00	Demo: Introduction to experimental set-ups of day 1; microscope software for hardware control and image acquisition
14:00 – 16:00	Practical: Working at LSM and Imaging systems (handling, alignment and troubleshooting); Working with resolution test target and live cells; Recording of first 3d series
16:00 – 16:15	Break: Coffee and Tea
16:15 – 18:00	Practical: continued (different setup)

Day 2: April 17th, 08:30 – 18:00	
08:30 – 10:30	Lecture: Fluorescence microscopy, CCD-cameras, confocal microscopy, point spread function, pinhole settings, 2D / 3D acquisition parameters
10:30 – 10:45	Break: Coffee and Tea
10:45 – 12:00	Lecture: Dyes, Nanodots, optical filters, fluorescence spectra, spectral recording and special microscopy (2- photon microscopy, Nipkow based confocal, ApoTome, META-detector, Live, TIRF)
12:00 – 13:00	LUNCH BREAK
13:00 – 14:00	Demo: Introduction to experimental set-ups of day 2; acquisition parameters, z-stack and multichannel recording, movies
14:00 – 16:00	Practical: Recording of bead images on conventional and confocal microscopes; Factors regarding image quality, confocality, binning, averaging, summing, pixel time, illumination time, 3D stacks, z-distance; Recording of images and spectral information from fixed cells
16:00 – 16:15	Break: Coffee and Tea
16:15 – 18:00	Practical: continued (different setup)

Day 3: April 18th, 08:30 – 19:00	
08:30 – 10:30	Lecture: GFP-Methods (FRAP, FRET, FLIP, FLIM, FLAP)
10:30 – 10:45	Break: Coffee and Tea
10:45 – 12:00	Lecture: Fluorescence live-cell labelling, cell volume measurements, microinjection, flash-labelling, caged dyes, dye loading, ion-sensitive dyes, ratio-dyes, combination of dyes
12:00 – 13:00	LUNCH BREAK
13:00 – 14:00	Demo: Introduction to experimental set-ups of day 3; recording spectra, linear unmixing, time-lapse recording, multidimensional recordings, how to FRAP / FRET, ratio recordings & analysis
14:00 – 15:30	Practical: Live-cell experiments LSM-I-NLO: FRET DUO Live: Photo-activation and –conversion Imaging 3: Ca measurement Imaging 4: TIRF and 4d time lapse
15:30 – 15:45	Break: Coffee and Tea
15:45 – 17:15	Practical: continued (different setup)
17:15 – 19:00	Analysis: Making first image montages; Quantitative analysis of images: intensity, area, S/N....

Day 4: April 19th, 08:30 – 19:00	
08:30 – 10:30	Lecture: Superresolution, Deconvolution
10:30 – 10:45	Break: Coffee and Tea
10:45 – 12:00	Demo: Analysis of time series, batch analysis of images, 3D and 4D software (Imaris), movies with ZEN, Macromedia and VideoMach
12:00 – 13:00	LUNCH BREAK
13:00 – 15:00	Practical: Live-cell experiments LSM-I-NLO: FRET DUO Live: Photoactivation and –conversion Imaging 3: Ca measurement Imaging 4: TIRF and Time lapse in 4D
15:00 – 15:15	Break: Coffee and Tea
15:15 – 17:15	Practical: continued (different setup)
17:15 – 19:00	Analysis: Analysis of experiments, generating movies and diagrams

Day 5: April 20th, 08:30 – 16:00	
08:30 – 10:00	Demo: generating movies with ZEN, VideoMach3D and Imaris; Deconvolution with Huygens
10:00 – 10:15	Break: Coffee and Tea
10:15 – 12:00	Analysis: Data analysis, generating animations and collages; Preparation of presentation
12:00 – 13:00	LUNCH BREAK
13:00 – 15:00	Analysis: continued
15:00 – 16:00	Data presentation in groups, wrap-up and feedback

- Please note that this preliminary agenda is for your information only and subject to changes.
- For more detailed information, please contact MIAP: <https://www.miap.eu> info@miap.eu

