

## 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.

**November 13<sup>th</sup> – 17<sup>th</sup> 2017**

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:** Dr. Roland Nitschke, Iris Bierschenk, Elitsa Bodurova, Sabine Haxelmans, Dr. Angela Naumann, Tobias Wernet

Day 1: November 13 <sup>th</sup> , 08:30 – 18:00	
08:30 – 09:15	<b>General introduction; Overview of imaging methods</b>
09:15 – 10:30	<b>Lecture:</b> 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	<b>Break: Coffee and Tea</b>
10:45 – 12:00	<b>Lecture:</b> Immunofluorescence labelling, sample preparation, cover glasses, mounting media and common pitfalls
12:00 – 13:00	<b>LUNCH BREAK</b>
13:00 – 14:00	<b>Demo:</b> Introduction to today's experimental set-ups; microscope software for hardware control and image acquisition
14:00 – 16:00	<b>Practical:</b> 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	<b>Break: Coffee and Tea</b>
16:15 – 18:00	<b>Practical:</b> continued (different setup)

  

Day 2: November 14 <sup>th</sup> , 08:30 – 18:00	
08:30 – 10:30	<b>Lecture:</b> Fluorescence microscopy, CCD-cameras, confocal microscopy, point spread function, pinhole settings, 2D / 3D acquisition parameters
10:30 – 10:45	<b>Break: Coffee and Tea</b>
10:45 – 12:00	<b>Lecture:</b> 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	<b>LUNCH BREAK</b>
13:00 – 14:00	<b>Demo:</b> Introduction to today's experimental set-ups; acquisition parameters, z-stack and multichannel recording, movies
14:00 – 16:00	<b>Practical:</b> 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	<b>Break: Coffee and Tea</b>
16:15 – 18:00	<b>Practical:</b> continued (different setup)

Day 3: November 15 <sup>th</sup> , 08:30 – 19:00	
08:30 – 10:30	<b>Lecture:</b> GFP-Methods (FRAP, FRET, FLIP, FLIM, FLAP)
10:30 – 10:45	<b>Break: Coffee and Tea</b>
10:45 – 12:00	<b>Lecture:</b> 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	<b>LUNCH BREAK</b>
13:00 – 14:00	<b>Demo:</b> Introduction to today's experimental set-ups; recording spectra, linear unmixing, time-lapse recording, multidimensional recordings, how to FRAP / FRET, ratio recordings & analysis
14:00 – 15:30	<b>Practical:</b> 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	<b>Break: Coffee and Tea</b>
15:45 – 17:15	<b>Practical:</b> continued (different setup)
17:15 – 19:00	<b>Analysis:</b> Making first image montages; Quantitative analysis of images: intensity, area, S/N....

Day 4: November 16 <sup>th</sup> , 08:30 – 19:00	
08:30 – 10:30	<b>Lecture:</b> Superresolution, Deconvolution
10:30 – 10:45	<b>Break: Coffee and Tea</b>
10:45 – 12:00	<b>Demo:</b> Analysis of time series, batch analysis of images, 3D and 4D software (Imaris), movies with ZEN, Macromedia and VideoMach
12:00 – 13:00	<b>LUNCH BREAK</b>
13:00 – 15:00	<b>Practical:</b> 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	<b>Break: Coffee and Tea</b>
15:15 – 17:15	<b>Practical:</b> continued (different setup)
17:15 – 19:00	<b>Analysis:</b> Analysis of experiments, generating movies and diagrams

Day 5: November 17 <sup>th</sup> , 08:30 – 16:00	
08:30 – 10:00	<b>Demo:</b> generating movies with ZEN, VideoMach3D and Imaris; Deconvolution with Huygens
10:00 – 10:15	<b>Break: Coffee and Tea</b>
10:15 – 12:00	<b>Analysis:</b> Data analysis, generating animations and collages; Preparation of presentation
12:00 – 13:00	<b>LUNCH BREAK</b>
13:00 – 15:00	<b>Analysis:</b> continued
15:00 – 16:00	Data presentation of groups, wrap-up and feedback

- Please note that this preliminary agenda is subject to changes. The final agenda will be released prior to the workshop.
- We will use the LIC workstations for image and data analysis. However, you can bring your own laptop (Wifi connection available) with own software licenses.
- For more information, please contact MIAP: <http://www.miap.eu> [info@miap.eu](mailto:info@miap.eu)

