Modern Advanced Confocal Microscopy

Theoretical and practical insights into CLEM, STED super-resolution, expansion microscopy and multiplexing

supported by the PhD specialization Cutting Edge Microscopy, www.mic.unibe.ch

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BIOS+, via Francesco Chiesa 5, Bellinzona – Room 1st floor South West

Summary

Confocal microscopy has become a cornerstone technology in modern life sciences. Today, confocal microscopy serves not only as a powerful standalone imaging modality but also as a versatile platform that integrates with advanced techniques to push the limits of biological discovery. Modern approaches, such as Correlative Light and Electron Microscopy (CLEM), STED super-resolution and expansion microscopy can extend beyond the classical diffraction limit. Meanwhile, multiplex confocal imaging enables the simultaneous detection of numerous molecular markers in 3D samples. This course will explore the theoretical basis and provide a practical overview of the mentioned techniques.

This is an **advanced course**: basic theoretical knowledge (for example from user trainings or microscopy course) and solid practical experience of confocal microscopy are required. Participation is limited to 10 people. All submitted applications will be screened by organizers based on the applicant's abstract, which should outline candidate's interest in the course, including their goals and how they hope to benefit. Priority will be given to PhD students. Click here to submit your application (deadline Aug 1st). This course awards 0.5 ECTS.

Program outline

Morning 9:30-12:00 (30 min break). Location: Room 1st floor South West

Welcome and introduction round

Principles of confocal microscopy, resolution, sampling. Detector types and noise. Detector modes (counting or analog) and applications. Laser types and the WLL. Multiplex imaging with optimization of channel use, lambda scans, cyclic imaging. Classical whole-mount staining and imaging protocols.

Principles of STED microscopy and resolution improvement beyond the diffraction limit. Dye selection for efficient depletion and optimal signal. Antibody size and protein dimensions in relation to achievable resolution. Refractive index matching and coverslip thickness for minimizing aberrations. Channel separation strategies in multicolor STED imaging.

CLEM workflows for RT-EM and connection with super-resolution techniques. Expansion microscopy workflow. Chemical fixation artifacts.

Deconvolution demo. Improvement of low SNR for resonance scanner/live cell imaging and STED deconvolution for further resolution improvement.

Afternoon 13:30-17:30 (30 min break). Location: two-group rotation at microscopy rooms 043 and 045

Rotation 1. Stellaris 8 STED FALCON. Goal: Improve SNR and resolution.

Rotation 2. Stellaris 5. Goal: CLEM and spectral unmixing.

Course wrap up and student feedback.