Advanced Imaging
Spring Semester 2024
(SBL.00419)

Description:
Fluorescence microscopy has become the preferred imaging tool for biological systems due to its capability to visualize specifically the target biomolecules under conditions compatible with life, like room temperature, liquid environment and irradiation with visible light. Fluorescence microscopy also provides very high sensitivity down to the detection of single molecules. As drawback, as it happens with any a far-field optical technique, the spatial resolution is limited by the wavelength of light to a few hundreds of nanometres (the so-called diffraction limit). Remarkably, in the mid 2000s, a series of imaging methods using fluorescence readout were developed that deliver images with resolution beyond the diffraction limit. These methods, called super-resolution fluorescence microscopy or far-field fluorescence nanoscopy and whose pioneers were awarded with the Nobel Prize in Chemistry in 2014(*1–3*), have revolutionized biological imaging and continued to be developed until the present day.

In these lectures, we will revise the working principles of super-resolution microscopy and its development from the first generation of methods up to the newest methods capable of achieving 1 nm resolution under ambient conditions(*4–7*). We will discuss the performance and technical aspects of the necessary hardware and sample preparation for each one of the different modalities of super-resolution microscopy existing today. Also, we will perform experiments hands-on to obtain super-resolved fluorescence images, including the acquisition and analysis of data.

Learning objectives:
After this course, the student will know:

- The fundamental principles of super-resolution fluorescence microscopy in its different modalities: coordinate stochastic single-molecule localization microscopy (e.g., STORM, PALM, DNA-PAINT), coordinate targeted super-resolution microscopy (e.g., STED, RESOLFT), and single-molecule localization through sequential structured illumination (e.g., MINFLUX, RASTMIN).
- How to choose the best suited super-resolution method for specific applications: visualizing fixed or living specimens, compromise between resolution and throughput (image acquisition speed), visualizing structures or trajectories.
- How acquire single-molecule localization data and analyse it to obtain a super-resolved fluorescence image

Target audience:
Master of Science in Biology, Department of Biology, University of Fribourg
Doctoral students of the Fribourg Graduate School of Life Sciences and Medicine (FGLM)
Doctoral students of the MIC PhD programme in Cutting Edge Microscopy, University of Berne

Length Block Course 7hrs

Credits 1 ECTS

10/02/2024 be fs

Time and Location Spring term 2024

Thursday March 14th, 13.15-17h, PER21, Room B207
Friday March 15th, 13.15-17h, PER17, Room 001

Lecturer Fernando Stefani

Instructors Mariano Barella
Guillermo Acuna
Felix Meyenhofer
Caio Fabio Baeta Lopes
Boris Egger

Course Organiser Boris Egger

Grading Written Exam

References:

1. S. W. Hell, Nanoscopy with Focused Light (Nobel Lecture). *Angew. Chemie Int. Ed.* **54**, 8054–8066 (2015).
2. E. Betzig, Single Molecules, Cells, and Super-Resolution Optics (Nobel Lecture). *Angew. Chemie Int. Ed.* **54**, 8034–8053 (2015).
3. W. E. Moerner, Single-Molecule Spectroscopy, Imaging, and Photocontrol: Foundations for Super-Resolution Microscopy (Nobel Lecture). *Angew. Chemie Int. Ed.* **54**, 8067–8093 (2015).
4. F. Balzarotti, Y. Eilers, K. C. Gwosch, A. H. Gynnå, V. Westphal, F. D. Stefani, J. Elf, S. W. Hell, Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. *Science*. **355**, 606–612 (2017).
5. L. A. Masullo, A. M. Szalai, L. F. Lopez, F. D. Stefani, Fluorescence nanoscopy at the sub-10 nm scale. *Biophys. Rev.* **13**, 1101–1112 (2021).
6. L. A. Masullo, A. M. Szalai, L. F. Lopez, M. Pilo-Pais, G. P. Acuna, F. D. Stefani, An alternative to MINFLUX that enables nanometer resolution in a confocal microscope. *Light Sci. Appl.* **11**, 199 (2022).
7. P. Zdańkowski, L. F. Lopez, G. P. Acuna, F. D. Stefani, Nanometer Resolution Imaging and Tracking of Single Fluorophores by Sequential Structured Illumination. *ACS Photonics*. **9**, 3777–3785 (2022).