Summer School – Leica DMi8 S Fluorescence Microscope with 488 nm Laser Scanner

Microscopy in the context of cellular research is a time-honored, constantly evolving technique with broad applications. Typical applications are analysis and visualization of cellular kinetics, protein interactions and subcellular localization of proteins and protein complexes using e.g. fluorescent antibodies or genetically fused fluorescent proteins like the Nobel Prize-awarded Green Fluorescent Protein(GFP)-based method and its derivatives.

Camera-based widefield fluorescence microscopy is a fast, reliable technique to capture fluorescent images of cells, spheroids or organoids. The high sensitivity and short recording time enables high throughput with low phototoxicity and photobleaching at the same time, compared to laser scanning microscopes. A previous drawback especially with thicker specimens was the lower sharpness of the focal plane (Z-axis), known as out-of-focus blur. Enhanced Computational Clearing removes this blur in real time, creating confocal-like images of even large sample volumes. With this technique we can visualize more intracellular details, enabling more advanced, precise analyses and 3D reconstructions of samples.

The Leica DMi8 S Fluorescence Microscope is additionally equipped with a 488 nm Laser Scanner for light patterning and photo manipulation. This enables high-precision optical experiments in Optogenetics, light induced (de)activation of proteins, bleaching of fluorescent proteins and uncaging of chemical compounds. With this method you can control (bio)chemical systems - e.g. to bring a system out of thermodynamic equilibrium (relaxation experiments) to investigate phase separation.

We would like to offer a module to all interested parties that gives insight into the current possibilities of widefield fluorescence microscopy and which further experiments and questions of (not only) cell-based/biological systems can be additionally addressed with the equipped laser scanner.

Summer School – PicoQuant Fluorescence Lifetime Imaging Microscopy

Cells and organelles are delimited by lipid membranes. In a living cell the membrane shape changes constantly causing bending, shearing and stretching resulting in a change of the membrane tension. Membrane tension plays an essential role in numerous cellular (cell migration, spreading and division) and also subcellular processes (endocytosis and regulation of mechanosensitive ion channels) being therefore an important regulator in the living cell.

To probe changes in the membrane tension in biological processes, a non-invasive fluorescent probe can be applied and investigated by fluorescence-lifetime imaging microscopy (FLIM). FLIM is based on the differences on the excited state decay rate from a fluorescent sample. Being independent on e.g. concentration and absorption by the sample, the readout of the average time that a molecule remains in an excited state prior to returning to the ground state is more robust than in intensity-based methods. Detecting fluorescence lifetimes requires a high-quality optical setup with high-NA objectives and sensitive detectors allowing time-correlated single photon counting.

The planarizable push-pull probe FliptR targets plasma membrane in cells and also artificial membranes like unilamellar vesicles of different size. Changes in the membrane tension are reported through changes in its fluorescence lifetime (2,8-7ns). Upon pressure the lipid bilayers reorganize by undergoing phase separation leading to a twist in angle and polarization between the two twisted dithienothiophenes of the mechanophore.

Fluorescence-lifetime imaging is a powerful tool to not only detect changes in the local environment but also the detect molecular interactions, conformational changes and discrimination of multiple labels and background removal.