3D super-resolution imaging of cellular cytoskeleton

Laboratory of Biophotonics and Pharmacology, 74 route du Rhin F-67401 Illkirch

Until recently, optical microscopy was restricted by the diffraction phenomenon that limited the resolution to about 200 nm in the lateral direction and 500 nm in the axial direction. A critical improvement in the nanoscopic scale, especially in respect to intracellular imaging, has been achieved by the development of high-resolution microscopy, introducing the extremely promising era of “nanoscopy”. Recent advances in high-resolution fluorescence microscopy allowed breaking the diffraction limits of common optical microscopy, reaching resolution ≤50 nm. Both scanning fluorescence microscopy and widefield microscopy display developments in the nanoscopy field. Among these different nanoscopy techinques, we have developed a setup based on PhotoActivation Localization Microscopy (PALM) and we are currently building a STimulated Emission Depletion (STED) microscope. Both approaches allow to image nanoobjects within living cells with 30 nm resolution.

In the lab, we have developed a widefield TIRFM setup displaying single molecule sensitivity. The system is equipped with 5 cw lasers that can be used to perform 3D PALM or direct Stochastical Optical Reconstruction Microscopy (dSTORM). During the practical, the students will assist to an experiment in which fixed HeLa cells will be immunostained with Alexa 647 labeled antibodies directed against tubulin to visualize the cytoskeleton with an isotropic resolution of 40 nm.