Optical microscopy at the nanoscale

Alberto Diaspro

The course is aimed to provide a comprehensive approach to super-resolved optical microscopy methods towards biophysical studies at the nanoscale. Optical microscopy at the nanoscale is used to link single-molecule organization and behaviour in living cells with the global function of the cell. Advanced optical microscopy methods allow unlimited spatial resolution despite the diffraction limit related to the use of circular apertures and visible light. Image formation following optical data collection can take advantage of additional information that allows to circumvent the diffraction limit. Giuliano Toraldo di Francia analyzed such a possibility in terms of communication theory late in the forties. Eric Betzig, Stefan W.Hell and William E. Moerner received the Nobel prize for the development of super-resolved fluorescence microscopy in 2014. Different approaches to super-resolved fluorescence microscopy will be discussed, namely: single-molecule imaging, photo-activatable light microscopy (PALM), stimulated emission depletion microscopy (STED), expansion microscopy, individual molecule localization selected plane illumination microscopy (IML-SPIM) computational microscopy and structured illumination microscopy (SIM). Such microscopy methods will be integrated with multiphoton excitation and label-free approaches. Moreover, we will discuss the newest approach in advanced optical microscopy based on temporal and spatial information merged by the image scanning microscopy strategy. DNA and protein assemblies will be considered towards the biophysical understanding of oncological and neurodegenerative diseases.