Next generation localization microscopy - or - how and why to ruin a perfectly good microscope

Abstract:

In localization microscopy, the positions of individual nanoscale point emitters (e.g. fluorescent molecules) are determined at high precision from their point-spread functions (PSFs). This enables highly precise single/multiple-particle-tracking, as well as super-resolution microscopy, namely single molecule localization microscopy (SMLM). To obtain 3D localization, we employ PSF engineering, namely, we physically modify the standard PSF of the microscope, to encode the depth position of the emitter. In this talk I will describe how this method enables unprecedented capabilities in localization microscopy; specific applications include dense emitter fitting for super-resolution microscopy, multicolor imaging from grayscale data, volumetric multi-particle tracking/imaging, dynamic surface profiling, and high-throughput in-flow colocalization in live cells. We often combine the optical encoding method with neural nets (deep-learning) for decoding, i.e. image reconstruction; however, our use of neural nets is not limited to image processing - we use nets to design the optimal optical acquisition system in a task-specific manner.