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 \( \text{△} \) 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.