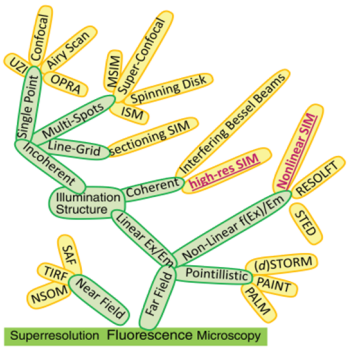
Seeing Is Believing

"It is very easy to answer many of these fundamental biological questions; you just look at the thing!"
Richard P. Feynman, "There's Plenty of Room at the Bottom," Caltech, December 29, 1959

This presentation is geared towards the latest developments in imaging platforms that are able to tackle biological samples. Visualizing living cells, single molecules, and even atoms is crucially important, but unfortunately excruciatingly difficult. Still, recent progress reveals that a wide variety of novel imaging techniques have reached maturity. We will recap here the principles behind techniques that allow imaging beyond the diffraction limit, and highlight both historical as well as fresh advances in the field of neuroscience (as a result of such imaging technologies). As an example, single-particle tracking is one of several tools able to study single molecules inside cells and reveal the dynamics of biological processes (receptor trafficking, signaling and cargo transport).

Historically, the first venture outside classical optics was represented by X-ray and electron based techniques. Out of these, electron microscopy allows higher resolution by far. In time this has diverged into TEM (transmission electron microscopy), SEM (scanning electron microscopy), REM (reflection electron microscopy), and STEM (scanning transmission electron microscopy), while lately these have started to merge with digital holography (scanning transmission electron holography, atomic-resolution holography, and low-energy electron holography). Electron microscopy allows resolutions down to 40pm, while it is not trivial to use such techniques on biological samples. The second departure from classical optics was represented by scanning probe techniques like: AFM (atomic force microscope), STM (scanning tunneling microscope), PFM (photonic force microscope), and RTM (recurrence tracking microscope). All of these rely on the physical contact of a solid probe tip which scans the surface of an object (which is supposed to be quite flat). The third attempt has come full circle, and is represented by super-resolution microscopy which won the Nobel Prize in 2014.



The presentation will start from basic principles, emphasizing the advantages and disadvantages of different bio-imaging techniques. The development of super-resolution microscopy techniques in the 1990's and 2000's (https://en.wikipedia.org/wiki/Super-resolution_microscopy) has allowed researchers to image fluorescent molecules at unprecedentedly small scales. This significant boost was properly acknowledged by replacing the term 'microscopy' with 'nanoscopy' which was coined by Stefan Walter Hell in 2007. It distinguishes novel diffraction-unlimited techniques from conventional approaches, e.g., confocal or wide-field microscopy. An incomplete list includes (among others): BALM (binding activated localization microscopy), COLD (cryogenic optical localization in 3D), fBALM (fluctuation-assisted BALM), FPALM (fluorescence photo-activation localization microscopy), GSDIM (ground state depletion microscopy), LSFM (Light sheet fluorescence microscopy), PALM (photo activated localization microscopy), SIM (structured illumination microscopy, including both linear and nonlinear), STED (stimulated emission depletion), STORM (stochastic optical reconstruction microscopy), SMLM (single molecule localization microscopy), SNOM (scanning near-field microscopy), and TIRF (total internal reflection fluorescence). Obviously, with such improvements in resolving power new avenues for studying synapses and neurons more generally are being opened, and a few of the latest experiments that highlight unique capabilities will be enumerated, briefly reviewed and compared.