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The Fundamentals of Single Molecule Microscopy.

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Abstract content
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In the last decade, single molecule microscopy has become the technique of choice when sub-diffraction imaging is required [1]. Sub-diffraction imaging using optical microscopes requires that single molecule fluorescent markers be imaged stochastically (in techniques like stochastic optical reconstruction microscopy (STORM) or photoactivated localization microscopy (PALM)) or with very low concentrations such that only a small fraction of the emitters are imaged at any given time [2]. When combining the abovementioned techniques with point-spread function tailoring, three dimensional localization accuracies well below 50 nm are readily achieved [3].

In this presentation the fundamental requirements for achieving high localization accuracies with single molecule microscopy will be discussed.

References:

[1] B. Flier, et. al. Heterogeneous Diffusion in Thin Polymer-Films as observed by High-Temperature Single Molecule Fluorescence Microscopy; J. Am. Chem. Soc. 2012, 134, 480-488.

[2] C. Galbraith, et al. Super-resolution microscopy at a glance; J Cell Sci. 2011, 124(10): 1607–1611.

[3] S. Pavani, et. al. Three-dimensional, single-molecule fluorescence imaging beyond the diffraction limit by using a double-helix point spread function; PNAS 2009, 106(9), 2995-2999.

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