OPTIMAL 3D AND MULTICOLOR LOCALIZATION MICROSCOPY
BY POINT SPREAD FUNCTION ENGINEERING

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Precise determination of the position of a single point source (e.g. fluorescent molecule, protein, quantum dot) is at the heart of microscopy methods such as single particle tracking\(^1\) and super-resolution localization microscopy ((F)PALM, STORM\(^2-4\)). Localizing a point source in all three dimensions (i.e. including depth) poses a significant challenge, since the depth of field of a standard high-NA microscope is fundamentally limited, and its point-spread-function (PSF), namely, the shape that a point source creates in the image plane, contains little information about the emitter’s depth. Various techniques exist that enable 3D localization, prominent among them being PSF engineering, in which the PSF of a microscope is modified to encode the depth (z position) of the source. This is achieved by shaping the wavefront of the light emitted from the sample, using a phase mask in the pupil (Fourier) plane of the microscope.

In this talk, I will describe how our search for the optimal PSF for 3D localization, using tools from estimation theory\(^5\), led to the development of microscopy systems with unprecedented capabilities in terms of depth of field\(^6\) and spectral discrimination\(^7\). Such methods enable fast, precise, non-destructive localization in thick samples and in multicolor. Applications of these novel advances will be demonstrated, including super-resolution imaging, tracking biomolecules in living cells and microfluidic flow profiling.

Figure 1. Three dimensional flow profiling using PSF engineering. (a) A single movie frame showing three fluorescent beads in water flowing in a microfluidic channel, imaged using a Tetrapod PSF. The shape of the PSF encodes the emitter’s depth over 20 µm. (b) Experimentally derived flow profile in channel, from the flowing-beads movie. (c,d) One dimensional slices from (b). Adapted from ref. 6.
References:


