Localizing and Tracking Single Nanoscale Emitters in Three Dimensions with High Spatiotemporal Resolution Using a Double-Helix Point Spread Function

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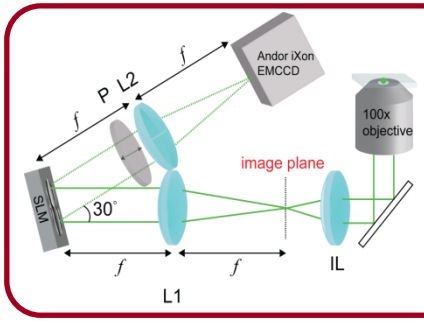
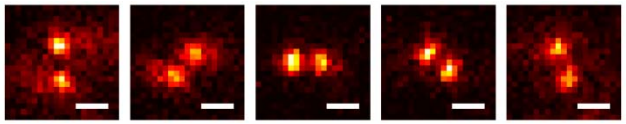
Expanding to Three Dimensions



The standard PSF of a microscope can be convolved with the DH-PSF by using a 4*f* optical system with a spatial light modulator (SLM) in the Fourier plane. A specially designed phase mask on the SLM creates the DH-PSF. The DH-PSF rotates as a function of the *z* position of the emitter, yielding a 2 μm depth of field.

Images of the DH-PSF as a function of *z*



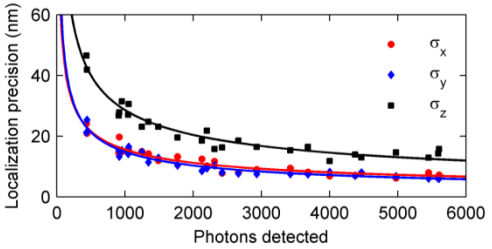
-1 μm -0.5 μm 0 μm 0.5 μm 1 μm

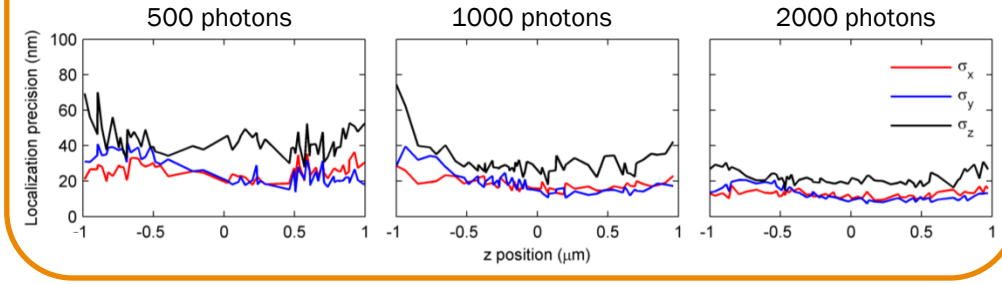
Scale bar is 1 μm

Localization Precision of the DH-PSF as a Function of Photons Detected and *z* Position of the Emitter

Measurements of the localization precision show that the DH-PSF can obtain 12 nm precision in *x*-*y* and 20 nm precision in *z* with 2000 photons detected. The localization precision stays relatively constant over a wide range of *z* values.

Best fit	*N*=500	*N*=2000
$\sigma_x = \frac{410}{N^{0.47}}$	22 nm	12 nm
$\sigma_y = \frac{550}{N^{0.52}}$	22 nm	11 nm
$\sigma_z = \frac{829}{N^{0.49}}$	39 nm	20 nm

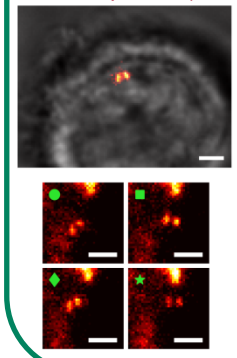




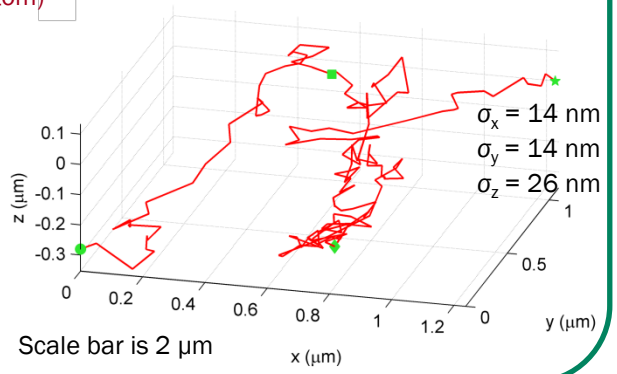
Tracking a Quantum Dot-Labeled Structure in a Live Cell in Three Dimensions

The DH-PSF successfully tracked the movement of a quantum dot-labeled structure in three dimensions inside a live COLO205 cell (~8-10 μm in diameter). This shows that the DH-PSF is excellent for *in vivo* tracking and robust against imaging aberrations associated with thick live cells.

White light image (top); DH-PSF fluorescence at various time points (bottom)



Three dimensional track of quantum dot-labeled structure



Scale bar is 2 μm

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