Vortex PSF for simultaneous 3D position and orientation localization microscopy

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We have developed a Point Spread Function (PSF) engineering approach that encodes the azimuthal and polar orientation of constrained dipole emitters together with its 3D position and degree or rotational constraint into a single in-focus image. This new PSF, which we call the Vortex PSF, enables the use of a less experimentally complex setup and a more compact PSF for orientation estimation compared to other extensive optical setups that also separate polarization in the emission path [1]. With a standard non-engineered PSF, the shape does not vary a lot as a function of the dipole orientation. In addition, large position biases can occur with defocus due to the directional emission of polarized light. For the Vortex PSF a spiral phase plate is inserted in the Fourier plane of the imaging system (Figure 1A), breaking the symmetry that the standard PSF has. This gives rise to a PSF that varies more as a function of the dipole orientation, as illustrated in Figure 1B. Along with an accurate model and efficient estimator we can extract the 3D position, orientation, degree of rotational constraint and signal/background photon counts from a single image. A CRLB precision of $\sigma_{xy} = 5.6$ nm, $\sigma_z = 27$ nm, $\sigma_{azimuthal} = 5.5°$, and $\sigma_{polar} = 3.1°$ can be achieved with 4000 signal photons.

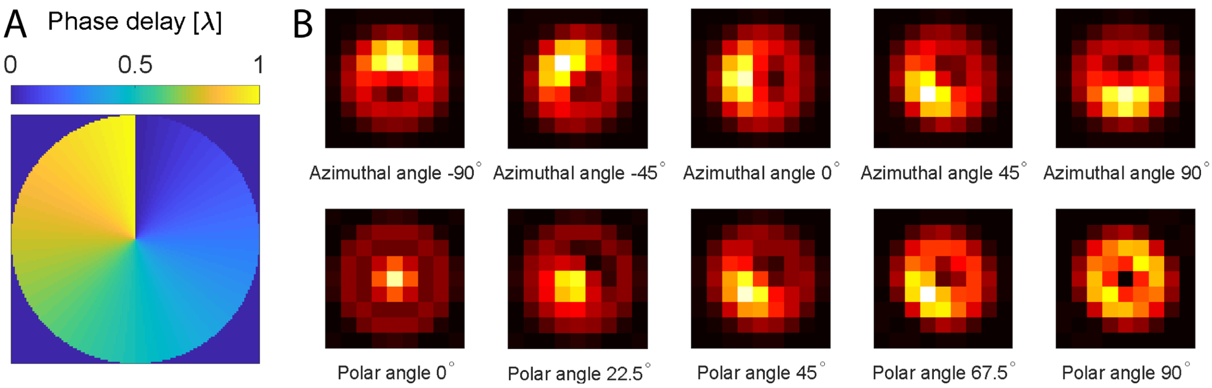

Figure 1: **A**) The phase plate in the Fourier plane applies a $\pi/2$ phase shift between radially opposing regions. **B**) In-focus PSFs of fixed dipole emitters using the Vortex PSF. Top row has a fixed polar angle of 45 degrees and the azimuthal angle is varied from -90 to 90 degrees. Bottom row has a fixed azimuthal angle of 45 degrees and the polar angle is varied from 0 to 90 degrees. (80 nm pixel size)

In our presentation we will explain the principle in detail, show its strengths compared to alternative methods, and show validation experiments of fixed single-molecule emitters. Furthermore we will showcase two applications; tracking the reorientation of single-molecules adhered to a surface and a full exploration of the binding landscape of λ-DNA using Binding-Activated Localization Microscopy (BALM).

[1] A.S. Backer, M.P. Backlund, M.D. Lew, W. E. Moerner, "Single-molecule orientation measurements with a quadrated pupil", *Optics Letters* **38**: 1521-1523 (2013)