

OPTICS FOR PSF ENGINEERING

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1. PSF ENGINEERING

Point Spread Function (PSF) engineering is a powerful tool for super resolution microscopy. For example STED uses a vortex phase plate to make donut-shaped spot, and double helix (DH) PSF phase mask was invented for precise fluorescence molecule 3D localization [1]. Usually these masks are designed to be set at pupil plane or its conjugate plane of objective lens because pupil plane is the Fourier plane.

2. REQUIREMENTS FOR OPTICS

Typically, the exit pupil of microscope objective lens is inside of the lens module. To use PSF engineering, the exit pupil should be projected outside of the lens using image relay optics. This optics should have diffraction limited performance and the addition aberration introduced by the pupil relay optics should be negligible. However, if we use commercially available lenses for the relay optics, it is very hard to guarantee the performance since we can get only lens design data, which does not include manufacturing and assembling tolerances. We designed and assembled custom optics considering tolerances to achieve diffraction limited performance and to minimize pupil aberration (Hamamatsu W-VIEW GEMINI-2C). The relay optics is equipped with a Bertrand lens for aligning the pupil position and phase mask. A field lens is used to adapt different microscopes.

3. EXPERIMENTAL RESULTS

The optics showed diffraction limited performance with wavefront aberration of less than 0.2λ excluding tube lens. We imaged $0.2\mu\text{m}$ fluorescence beads using a double helix phase mask at different image heights (0 and 6 mm) using a 60X NA1.4 objective lens. The images of the beads showed the same shape at each image height (Figure 1), enabling high 3D localization precision and accuracy

References

[1] Pavani, S. R. P., M. A. Thompson, W. E. Moerner. 2009. Three dimensional, single-molecule fluorescence imaging beyond the diffraction limit by using a double-helix point spread function. Proc. Natl.Acad. Sci. USA. 106:2995–2999.

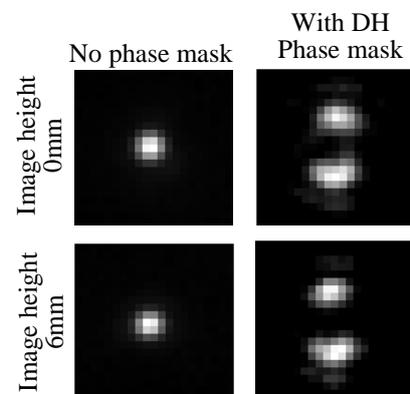


Figure 1: Images of the PSF