Supplementary information

Breaking the diffraction-limited resolution barrier in fiber-optical two-photon fluorescence endoscopy by an azimuthally-polarized beam

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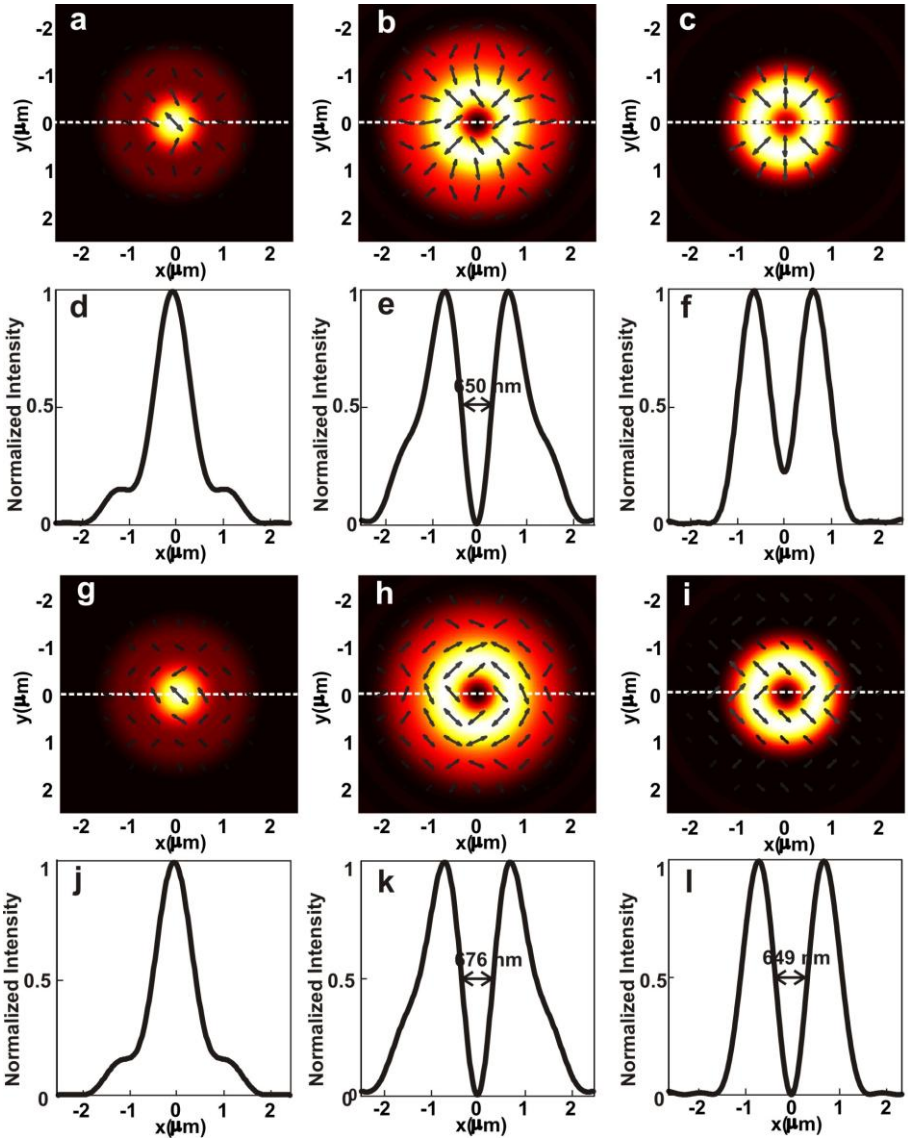
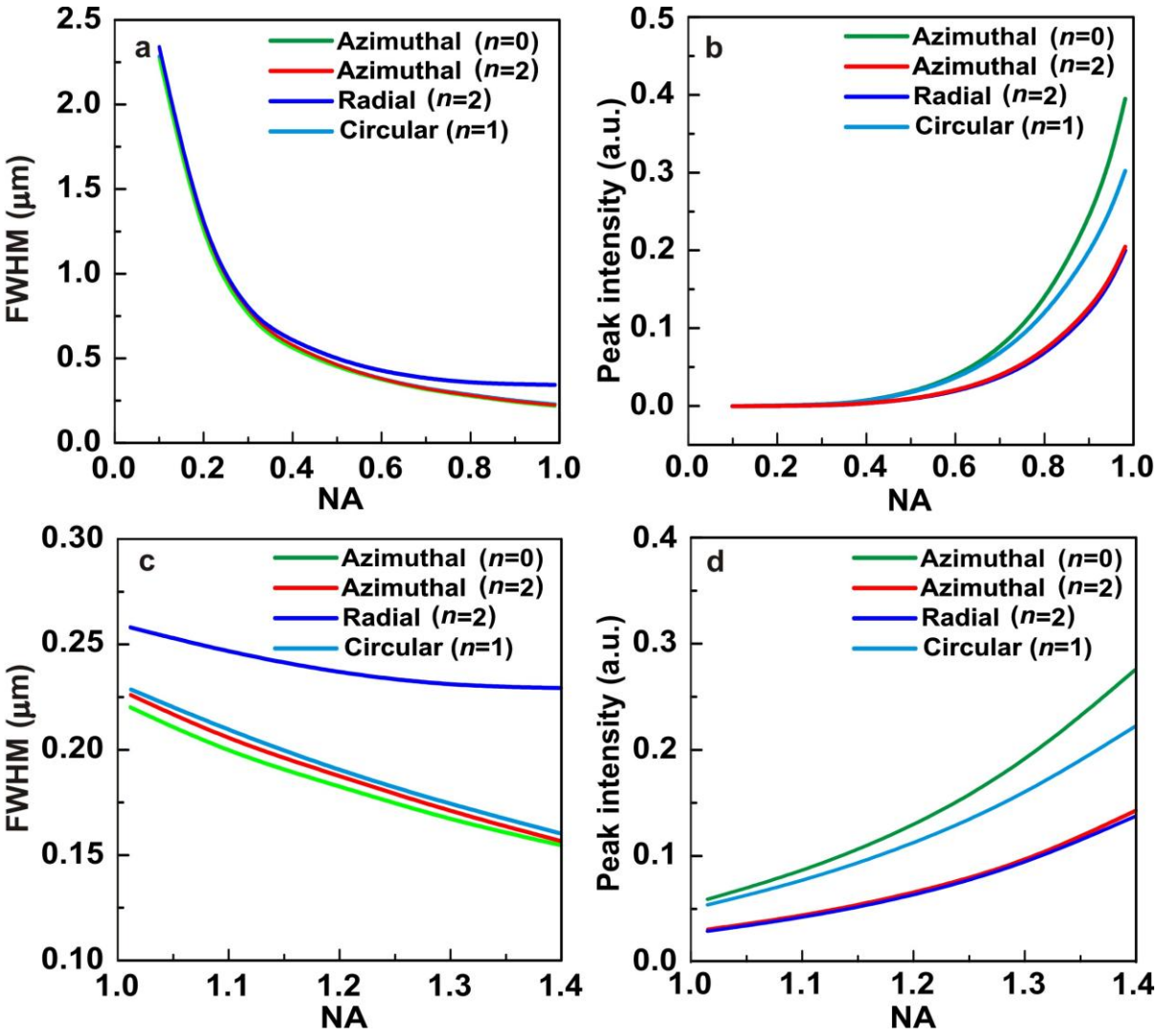
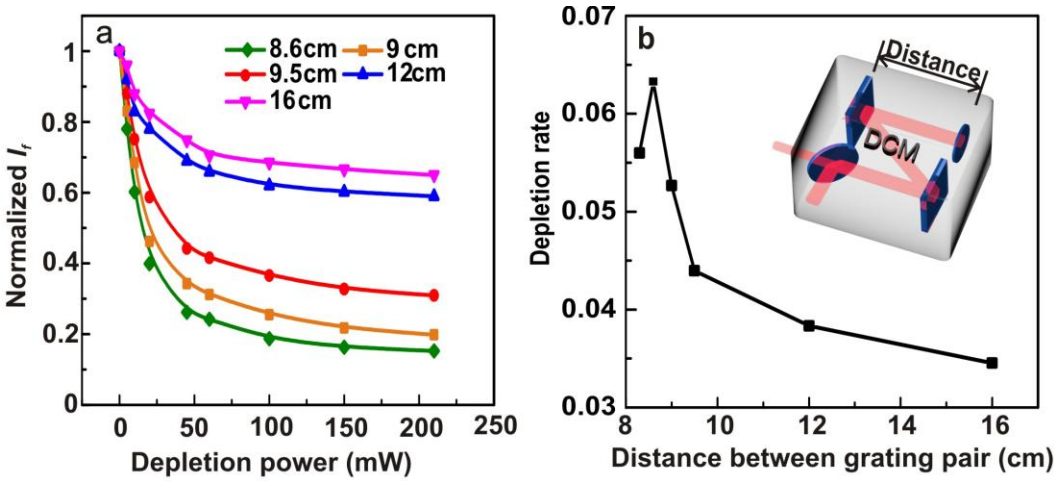
Supplementary Figures



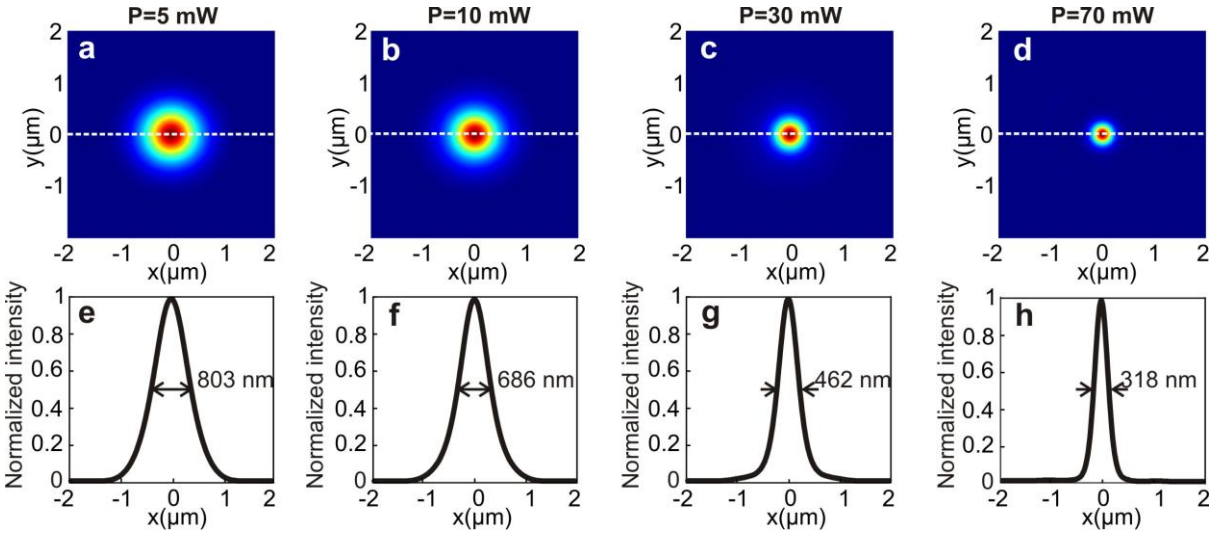
Supplementary Figure S1. Intensity distribution in the focal plane for an objective with NA = 0.35. (a) and (b) Azimuthally-polarized vortex beam with topological charges 1 and 2, respectively. (c) Radially-polarized beam without vortex. (d) - (f) Cross-sections corresponding to (a)-(c). (g) and (h) Radially-polarized vortex beam with topological charges 1 and 2, respectively. (i) Left-handed circularly-polarized vortex beam with topological charge 1. (j)-(l) Cross-sections corresponding to (g)-(i). The arrows present the electric field vectors projected on the *x*-*y* plane at $z = 0$.



Supplementary Figure S2. (a) and (b) Effect of NA on the FWHM and the peak intensity in the focal plane under the illumination of azimuthally- and radially-polarized beams without and with topological charge 2, and a circularly-polarized beam with topological charge 1 for a dry objective (NA <1). (c) and (d) Effect of NA on the FWHM and the peak intensity in the focal plane under the illumination of azimuthally- and radially-polarized beams without and with topological charge 2, and a circularly-polarized beam with topological charge 1 for a water-immersion objective with NA ranging from 1.0 to 1.4 at a coverglass/water interface (n_1/ n_2 = 1.515/1.33).



Supplementary Figure S3. Optimization of the depletion rate. (a) Normalized fluorescence intensity as a function of the depletion power for different distances between the grating pair. (b) The dependence of the depletion rate on the distance between the grating pair. Inset: Schematic of the DCM composed of a grating pair.



Supplementary Figure S4. The calculated intensity distribution of the point spread function for the fiber-optical STED two-photon endoscope in the focal region for NA = 0.35 at the depletion beam power of 5 mW (a), 10 mW (b), 30 mW (c) and 70 mW (d). (e)-(h) are cross-sections of the intensity distributions corresponding to (a)-(d).

Supplementary Discussion

Effect of the numerical aperture on the doughnut focal spots. Although no obvious difference of the FWHM and the peak intensity for an azimuthally-polarized beam, circularly-polarized vortex beam (n = 1), an azimuthally-polarized vortex beam (n = 2) and radially-polarized vortex beam (n = 2) for NA smaller than 0.4 (Supplementary Figs. S2a and S2b), an azimuthally-polarized beam always possesses the smallest FWHM and the strongest peak intensity in the focal region compared to the other two beams. As NA increase up to 0.98, the ratios of the FWHM and the peak intensity for an azimuthally-polarized beam to those in the other three cases are 0.63:0.69:0.63:1 and 1.98:1.51:1.02:1, respectively. For NA = 1.2, the ratios become 0.7:0.73:0.73:1 and 1.99:1.64:1.03:1, respectively (Supplementary Figs. S2c and S2d). Thus, a stronger peak intensity can be observed under the illumination of an azimuthally-polarized beam.

Effect of the pulse width of the excitation beam on depletion efficiency. In our experiment, a grating pair is adopted to pre-compensate for the chromatic dispersion. By properly adjusting the distance between the two gratings, the pulse width at the distal end of the DCF can be tuned. Fluorescent microspheres (Molecular Probes) with the fluorescence peak at wavelength 525 nm were selected as sample because the wavelength of 592 nm is located at the red-shifted tail of the fluorescence spectrum, which can achieve the effective depletion of population on the excited energy level. To experimentally verify the feasibility of the fluorescence microspheres in the STED imaging, we conduct the depletion experiments with fundamental modes of both the excitation and the depletion beams propagating through the core of the DCF. By overlapping these two beams in the focal region, the fluorescence intensity exhibits an exponential decay with the depletion power (Supplementary Fig. S3a). It is also interesting to note that the fluorescence can be depleted most efficiently at the optimized pulse width (i.e. the distance between the grating pair). The dependence of the fluorescence depletion rate on the distance between the two gratings is depicted in Supplementary Fig. S3b. It can be seen that the largest depletion rate is 0.064 when the distance between two gratings is 8.6 cm.