ENHANCED SPATIAL RESOLUTION BY SUBTRACTION IMAGING USING HIGHER-ORDER VECTOR BEAMS

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Subtraction imaging [1] is a method for effectively improving the spatial resolution in laser scanning microscopy, which is achieved by the subtraction between two images taken by two different point spread functions (PSFs) with bright and dark spots, respectively. Despite a relatively simple and intuitive principle, the spatial resolution of images can be greatly enhanced owing to the smaller center hole produced by a dark spot PSF.

Recently, we have numerically revealed that the subtraction between two PSFs generated by precisely designed vector beams, which have inhomogeneous polarization patterns on the beam cross-section, has the potential to achieve the spatial resolution approaching sub-100 nm even in conventional confocal laser scanning microscopy (CLSM) [2]. Here, we develop a laser scanning microscope that utilizes two different vector beams producing a flat-top PSF and a smaller dark spot PSF, respectively. In this setup, the spatial resolution close to 100 nm is indeed demonstrated experimentally by this simple subtraction.

A 532 nm laser beam employed as an excitation beam was focused by an oil-immersion objective with a numerical aperture (NA) of 1.45. Confocal images with a pinhole of 0.5 Airy units were acquired by two PSFs. One is a dark spot PSF with a small center hole produced by a higher-order azimuthally polarized beam, which has a quadruple-ring-shaped intensity distribution. The full-width at half-maximum (FWHM) value of the center dark spot PSF was calculated to be 92 nm. To take full advantage of such a small dark spot in subtraction imaging, we used another PSF with a flat-top shape obtained by a superposition of radially and azimuthally polarized beams. Those vector beams were generated by using specially designed, transmissive liquid crystal devices. By using this setup, the lateral spatial resolution in confocal imaging was successfully enhanced enough to clearly distinguish each bead as shown in Fig. 1. The FWHM values of the PSF was estimated to be ~100 nm for the resultant image of an isolated fluorescent bead with a diameter of 100 nm. This simple subtraction procedure can be applicable to many conventional and commercially-available laser microscopes.

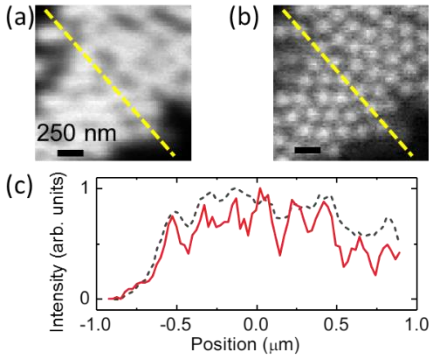

Fig. 1. Measured images of fluorescent beads (170 nm) by (a) conventional CLSM and (b) subtraction imaging. Intensity profiles along the lines in (a) and (b) are shown in (c).

[1] H. Dehez *et al*., "Resolution and contrast enhancement in laser scanning microscopy using dark beam imaging," *Opt. Express* **21**, 15912-15925 (2013).
[2] S. Segawa *et al*., "Resolution enhancement of confocal microscopy by subtraction method with vector beams," *Opt. Lett.* **39**, 3118-3121 (2014).