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Super-resolution microscopy based on fluorescence emission difference of cylindrical vector beams

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ABSTRACT

We propose a novel fluorescence emission difference microscopy (FED) system based on focusing cylindrical vector beams. In conventional FED, a Gaussian beam and a 0–2π vortex phase plate are used to generate solid and hollow spots. We focus radially polarized and azimuthally polarized cylindrical vector beams to obtain an expanded solid spot and a shrunken hollow spot, taking advantage of the optical properties of cylindrical vector beams to improve the conventional FED performance. Our novel method enhances FED performance because the hollow spot size determines the FED resolution and an expanded solid spot effectively reduces negative side-lobe emergence during image processing. We demonstrate improved performance theoretically and experimentally using an in-house built FED. Our FED achieved a resolution of less than $\lambda/4$ in test images of 100 nm nanoparticles, better than the confocal image resolution by a factor of approximately 1/3. We also discuss detailed simulation analyses and FED imaging of biological cells.

1. Introduction

Owing to its simplicity, versatility, and noninvasiveness, far-field fluorescence microscopy has long been applied in biological and medical science to observe and investigate microstructures and their movements [1]. However, the diffraction barrier [2] confines the resolving ability of conventional far-field fluorescence microscopy above about half the illumination light wavelength, restricting observation of microstructures featuring length scales less than 100 nm, such as vimentin fibers [3], vesicles, and microtubules. Confocal scanning laser microscopy (CSLM) [4] can enhance spatial resolution by a factor of $\sqrt{2}$ and improve micrograph contrast by spatial filtering with a pinhole, which also endows CSLM with optical sectioning ability [5]. Nonetheless, CSLM resolution is still limited by the diffraction barrier.

Fluorescence emission difference (FED) [15,16] microscopy was recently reported as a novel super-resolution technique based on intensity subtraction [17] between two images acquired under different illumination patterns. It joins a long list of validated super-resolution fluorescence microscopy methods, e.g., photo-activated localization microscopy (PALM) [6], stochastic optical reconstruction microscopy (STORM) [7], stimulated emission depletion microscopy (STED) [8], structured illumination microscopy (SIM) [9], super-resolution optical fluctuation imaging (SOFI) [10], total internal reflection fluorescence microscopy (TIRF) [11], and others [12–14]. Super-resolution techniques can be classified into two categories according to underlying principles and resolving abilities: diffraction unlimited and limited. Diffraction-unlimited techniques, including PALM, STORM, and STED, can yield resolution far beyond the diffraction barrier and overcome the barrier in true sense. Diffraction-limited techniques, including SIM, FED, SOFI, and TIRF, are fundamentally limited by the diffraction barrier. SIM, SOFI, and FED can enhance the resolution by a factor of 2 at most, while TIRF achieves super-resolution in the axial direction, but does not enhance lateral resolution.

Subtractive imaging was invented decades ago to enhance image resolution by subtracting confocal signals taken at different collection pinhole sizes under the same illumination pattern [18,19]. However, its signal-to-noise ratio (SNR) is relatively low since final resolution-enhanced image construction uses signals emitted from the periphery of the excitation spot. To overcome this, fluorescence emission difference microscopy (FED) [15] and switching laser mode microscopy (SLAM) [12,20] were proposed as subtractive imaging alternatives that apply different illumination patterns with the same collection pinhole size. In FED, the sample is alternately scanned by a solid excitation spot and a doughnut-shaped excitation spot [21], and central signals are extracted by eliminating peripheral signals through subtraction [22]. The resolution and SNR of FED can be further enhanced through beam modulation [23–25] to decrease the solid and hollow spot

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sizes. However, image subtraction inevitably produces negative side-lobes in the FED point spread function (PSF) under any subtractive factor, since the PSF profiles of the two illumination modes do not perfectly match [26]. This mismatch may introduce artifacts and deteriorate image quality. To reduce negative side-lobes and eliminate image deformation, the PSF profiles of the two illumination patterns were approximately matched by beam modulation [25,26]. Kobayashi et al. numerically studied the FED subtractive factor r, offering considerable selection criteria for it [27]. Moreover, image subtractive techniques can be combined with other super-resolution methods such as TIRF [28] and STED [17] to further enhance their performance.

Since FED is, in principle, built upon CSLM, its attainable resolution is determined by the size of the two micro-imaging mode PSFs, not the diffraction barrier [23]. Moreover, FED achieves image resolution beyond the diffraction barrier at low laser intensity and relatively high imaging speed in a simple and compact system, which undoubtedly adds to its availability in practical scientific research [29].

Cylindrical vector beams, i.e., radially polarized beams and azimuthally polarized beams, have attracted a lot of interest due to their unique polarization states and prominent beam properties, and have prospective applications in technologies such as laser cutting, particle acceleration and optical microscopy [30]. Research show that a radially polarized field can be focused to a spot size of 0.16λ, significantly smaller than 0.26λ for a linear polarized field [31]; an azimuthally polarized field can similarly be focused to a smaller spot size than the hollow spot created by modulating a linear polarized field through a 0–2π vortex phase plate. However, tightly focusing a radially polarized beam by a high NA objective lens enlarges the solid spot size because of a focal lateral doughnut-shaped component that broadens the entire radially polarized beam PSF. We establish a novel FED system that capitalizes on these features of cylindrical vector beams to enhance performance.

We established a cylindrical vector beam FED system in which a radially polarized beam and an azimuthally polarized beam create an extended solid spot and a shrunken hollow spot, respectively, at the focal plane by tight focusing. Simulations demonstrated significant performance enhancement by cylindrical vector beam FED and experiments achieved a resolution less than 120 nm.

2. Theory

In FED, two different confocal scanning images are required to obtain the final FED image: the confocal image is acquired under the solid excitation pattern and the negative confocal image is acquired under the hollow excitation pattern [15]. The excited fluorescence is filtered by a pinhole and detected by a photomultiplier tube (PMT) to form both images. The final FED super-resolution image is constructed by mathematical intensity subtraction of the two images:

$$I_{FED} = I_{solid} - r \times I_{hollow} \tag{1}$$

Here, I_{FED}, I_{solid}, and I_{hollow} are the normalized intensity distributions of the FED, confocal, and negative confocal images, respectively, and r is the subtractive factor. Negative intensity differences, which are inevitable after subtraction, are excluded from the final image for better image quality. The resolving ability of FED is determined by the PSFs of the two illumination modes, which can be described by the Debye integral [23],

$$\mathrm{E}(r_2, \varphi_2, z_2) = iC \int\int_{\Omega} \sin\theta \cdot A_1(\theta, \varphi) \cdot A_2(\theta, \varphi) \cdot \begin{bmatrix} p_x \\ p_y \\ p_z \end{bmatrix} \cdot \exp[\mathrm{i}f(\theta, \varphi)]$$
$$\exp\left[ikn\left(z_2 \cos\theta + r_2 \sin\theta \cos\theta\left(\varphi - \varphi_2\right)\right)\right] d\theta\, d\varphi \tag{2}$$

$\mathrm{E}(r_2, \varphi_2, z_2)$ is the electric field vector at the point (r_2, φ_2, z_2) in cylindrical coordinates relative to an origin at the focal point of the objective lens. (θ, φ) represents angular position on the wave front of the incident beam, where θ is the angle between the ray direction and the optical axis and φ is the azimuthal angle. Ω represents the effective incident aperture of the beam, $A_1(\theta, \varphi)$ is the amplitude function of the input light, and $A_2(\theta, \varphi)$ is the aberration function determined by the structure of the objective lens. $\left[p_x, p_y, p_z\right]^T$ is the polarization state of the incident beam and $f(\theta, \varphi)$ represents the phase modulation function applied to the input light. From Eq. (2), tuning the polarization state, phase distribution, and amplitude distribution can optimize the PSF size of both imaging modes to improve the FED performance [23].

We used cylindrical vector beams to generate two patterns of illumination. Unlike conventional linearly polarized beams or circularly polarized beams, cylindrical vector beams feature axisymmetric and anisotropic polarizations. Axisymmetric and anisotropic polarization have invariant angles between the electric field vector direction and the radial direction throughout the beam cross-section [32]. Fig. 1(a) and (b) shows that the electric field vector directions of radially polarized and azimuthally polarized beams are always parallel and perpendicular to the radial direction, respectively. These polarization properties cause a tightly focused radially polarized beam or azimuthally polarized beam to create a solid spot or hollow spot on the focal plane, respectively.

When concentrated by a high NA objective lens, radial polarization direction is deflected, generating a longitudinal electric field component. The lateral electric field component then coexists with a strong longitudinal electric field component at the focal area. The intensity distributions of both components' focal areas follow Eq. (2). The lateral electric component appears as a hollow spot with a central valley, and the longitudinal electric component presents as a solid spot with a central peak. The hollow lateral component expands the solid PSF wider than a Gaussian beam; this expansion reduces the negative values resulting from image subtraction. A characteristic that we do not utilize here is the high strength of the longitudinal electric component relative to the lateral one, and its smaller spot size relative to linearly or circularly polarized beams [31]; a sharper solid focal spot can be obtained by extracting the lateral component. The azimuthal polarization directions remain unchanged by focusing with a high NA objective lens, therefore no longitudinal electric component is generated. According to vector beam diffraction theory, the azimuthally polarized beam only possesses a lateral electric component at the focal area and forms a hollow spot smaller than a Gaussian beam modulated through a 0–2π vortex phase plate [30].

Our FED PSF is established by subtracting the hollow spot generated by the azimuthally polarized beam scaled by a subtractive parameter from the solid spot generated by the radially polarized beam, as in Eq. (1).

3. Simulation

We first demonstrate the resolving ability and performance enhancement of our cylindrical vector beam FED by simulating both the conventional FED PSF and the new cylindrical vector beam FED PSF. The conventional FED PSF is generated by subtracting a hollow excitation pattern modulated by a 0–2π vortex phase plate from a solid confocal excitation pattern of a focused

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