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Background suppression in confocal scanning fluorescence microscopy with superoscillations

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ABSTRACT

In this paper, we propose a simple, novel method to suppress background noise in confocal scanning fluorescence microscopy images with superoscillations. The proposed method retains high resolution, while completely removing superoscillation sidebands. Simulations of images from conventional confocal scanning microscopy, superoscillations, and the proposed method are presented, demonstrating its effectiveness.

1. Introduction

Due to the diffraction limit, the resolution of conventional optical microscopy in the far field is constrained to $0.61\lambda/NA$, where λ is the illumination wavelength and NA is the numerical aperture. Confocal scanning microscopy has been used for three-dimensional specimen analysis, and is widely used since it can acquire high-resolution optical images of a certain depth [1]. The use of confocal techniques has improved resolution by a factor of $\sqrt{2}$ [2,3]. As a result, confocal scanning microscopy has become a routine tool for studying samples in the life sciences. However, its spatial resolution is restricted to ~200 nm due to the diffraction limit under common experimental conditions [4]. Recently, the demand for higher spatial resolution in optical microscopy has promoted the development of novel super-resolution techniques capable of beating the diffraction limit. Several well-known microscopy techniques have been developed, such as stimulated emission depletion (STED) [5,6], stochastic optical reconstruction microscopy (STORM) [7,8], photoactivated localization microscopy (PALM) [9,10], structured illumination microscopy (SIM) [11,12], and fluorescence emission differencing (FED) microscopy [13,14]. These methods have achieved a spatial resolution of 100 nm or less. In STED microscopy, the specimen is illuminated with two beams. The first beam is an ordinary pump beam that excites electrons into a high-energy state, while the second beam is a doughnut-shaped simulated beam that can move electrons to non-luminous energy levels. A smaller spot can be obtained by matching the pump and simulated beams [15]. STORM and PLAM utilize special dyes for stochastic fluorescence, and can locate a single fluorescing emitter to enhance resolution. By illuminating a sample with a structured light pattern, SIM can encode undetectable high-frequency information into a detectable low-frequency band, thereby increasing imaging resolution by a factor of 2 [16]. FED microscopy can improve imaging performance by subtracting two images obtained in different conditions [17,18]. While microscopy systems using these techniques are commercially available and attractive for bio-imaging, obstacles remain, such as the requirement for intricate optical systems, special specimen preparation, expensive instruments, and time-consuming 3D image data processing. These obstacles limit the application of such microscopy systems. Therefore, the development of these technologies and the search for simpler super-resolution techniques remain important research topics.

A description for superoscillations begins with physics and mathematics [19–21], which have been introduced into microscopic imaging as a new approach to achieve super-resolution. Super-oscillation refers to a phenomenon when an oscillation of a band-limited function can be faster than its fastest Fourier components. It can be regarded as a superposition of low-frequency beams. Surprisingly, a super-oscillation can survive for a relatively long time before fading away due to its complex momenta [22], and can transfer high-frequency information further than several wavelengths. However, a major drawback is the inevitable existence of high-energy regions away from the superoscillation region, which leads to a tradeoff between the duration and the effective bandwidth of the superoscillation region [23]. When managed improperly, these sidebands have proven problematic as they spillover, drowning

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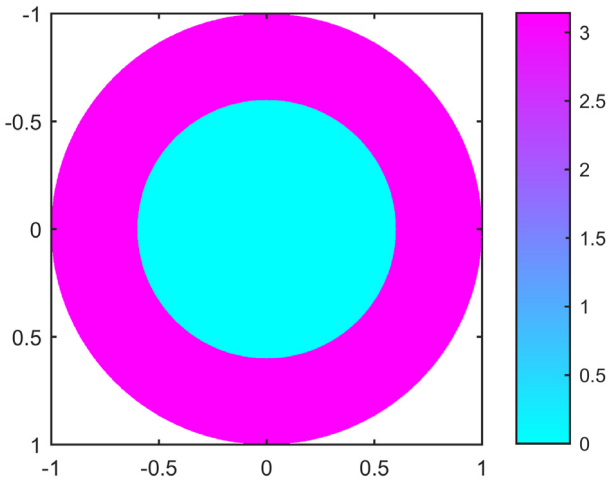

Fig. 1. π-phase mask used to generate superoscillation. The radius of the inside circle is $r2$ and the radius of the outside circle is $r1$.

out the superoscillating signal [24,25]. Previous studies have proposed different methods to manage sidebands and obtain superoscillation images. In some cases [26,27], the contribution of a superoscillation sideband to an image can be removed by a confocal imaging setup. In one approach sidebands have been moved away from the subwavelength hotspot to generate an appropriate window for imaging [28,29]. Studies also introduced superoscillations, demonstrating that their sidebands can remain at low energy for a weak subwavelength superoscillation hotspot [30,31]. A new design method has also been introduced to generate a diffraction-limited hotspot surrounded by low-energy superoscillating sidelobe ripples [32]. Other studies have introduced two photons to reduce superoscillation sidebands in confocal fluorescence microscopy. However, dealing with superoscillation sidebands remains a crucial consideration. In most cases, there are tradeoffs between resolution, viewing area, and/or sensitivity in the imaging system. In this paper, we propose a new method to remove superoscillation sidebands in confocal fluorescence microscopy. The proposed method retains a small superoscillation while removing sidebands.

In this paper, we present a novel method to suppress the background in scanning confocal fluorescence microscopy with superoscillations which is achieved by using subtraction. Section 2 describes the imaging theory of subtraction. Section 3 outlines simulation results and analysis. Conclusions are presented in Section 4.

2. Theory

The focal effects of incident light propagating through an objective lens can be calculated by vector diffraction theory. The electric field near the focus can be obtained by formulae which are explicitly derived from the Debye integral:

$$\vec{E}\left(r_2,\varphi_2,z_2\right) = iC\iint_{\Omega}\sin(\theta)\,E_0 A(\theta,\varphi)\,P \times e^{i\Delta a(\theta,\varphi)}e^{ikn\left(z_2\cos\theta+r_2\sin\theta cons(\varphi-\varphi_2)\right)}d\theta d\varphi \quad (1)$$

where $\vec{E}\left(r_2,\varphi_2,z_2\right)$ is the electric field vector at (r_2,φ_2,z_2) expressed in cylindrical coordinates, C is a normalized constant, E_0 is the amplitude function of the input light, $A(\theta,\varphi)$ is a 3×3 matrix related to the structure of the imaging lens, and P is Jose's vector of the incident light. $\Delta a(\theta,\varphi)$ is a parameter characterizing the phase delay generated by the phase plate.

A single π-phase mask which can be used for the superoscillation of the point spread function as shown in Fig. 1 [33]. Superoscillations and their sidebands depend on the factor $r2/r1$. For analysis, the numerical aperture of the objective lens in this paper is 1.4 and the sample is placed in the medium, whose index refraction is 1.518. Fig. 2 shows the point spread functions of conventional confocal microscopy and superoscillation, respectively, where the ratio $r2/r1$ is set to 0.75. From Fig. 2(b), the superoscillation produces a large point spread function (PSF) with a small central lobe. To show how the size of the central lobe decreases through the use of the method proposed here, intensity distributions resulting from it and from conventional confocal microscopy at the central lobe are shown in Fig. 3. The size of the central lobe in the proposed method is smaller than that from conventional confocal microscopy. The full width at half maximum (FWHM) from conventional confocal microscopy and the proposed method are 0.396λ and 0.206λ, respectively. This means that the FWHM of the proposed method is ~1.92× smaller than that of conventional confocal microscopy, but superoscillation is retained and a sideband appears. The superoscillation sideband would be partially reduced by a pinhole in scanning confocal fluorescence microscopy [33]. However, it is not completely removed and can cause image artifacts. This effect is clearly shown in a simulated image in the following.

The superoscillation image can be represented by

$$\begin{aligned} g &= o\otimes\left[h_{\text{illu}}\times\left(h_{\text{dete}}\otimes pinhole\right)\right] = o\otimes\left[\left(h_{\text{illu\_centre}}+h_{\text{illu\_outside}}\right)\right. \\ &\quad\left.\times\left(h_{\text{dete}}\otimes pinhole\right)\right] \\ &= o\otimes\left[h_{\text{illu\_centre}}\times\left(h_{\text{dete}}\otimes pinhole\right)\right] \\ &\quad + o\otimes\left[h_{\text{illu\_outside}}\times\left(h_{\text{dete}}\otimes pinhole\right)\right] = g_{\text{centre}}+g_{\text{outside}} \end{aligned} \quad (2)$$

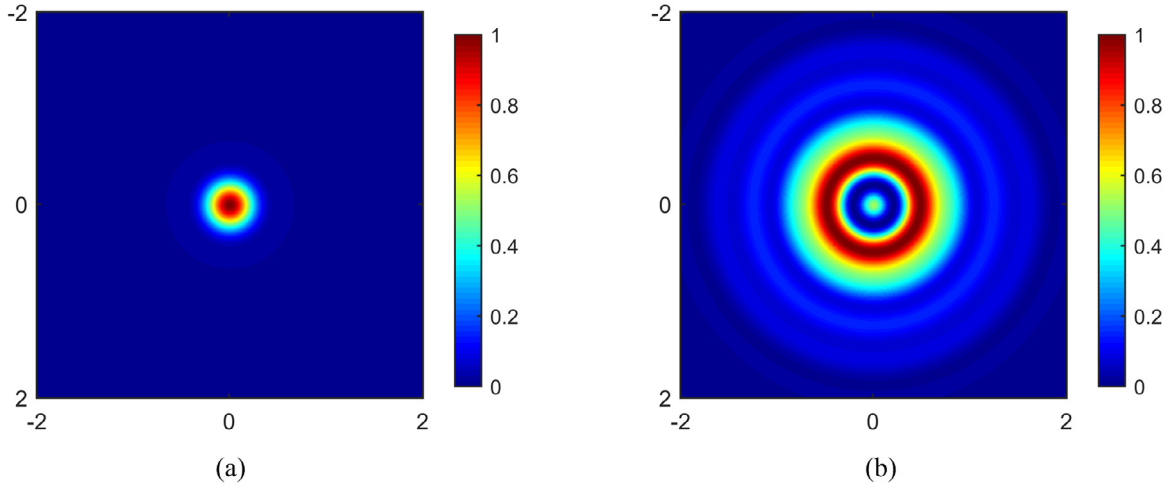

Fig. 2. Point spread functions from (a) conventional confocal microscopy and (b) superoscillation. Unit of axis is wavelength.