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Structured illumination fluorescence Fourier ptychographic microscopy

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ABSTRACT

We apply a Fourier ptychographic algorithm for fluorescent samples using structured illumination. The samples are illuminated with structured light patterns and the raw imaging data using traditional structured illumination fluorescence microscopy (SIM) are acquired. We then extract equivalent oblique illuminated images of fluorescent samples from the SIM images. An optimized Fourier ptychography algorithm is proposed, which ensures the fidelity of the reconstructed the super-resolution results. This method can break the diffraction limit to a resolution of $\lambda/4$, and has a better signal-to-noise ratio (SNR) than SIM, especially when the background noise is high.

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1. Introduction

In the past few decades, great efforts have been made toward developing super-resolution microscopy. Such efforts are motivated by the fact that the resolution of microscopy is not only limited by the diffraction limit, which is associated with the imaging system, but also by the sample. Against this background, we separate all current microscopy techniques into two types. The first type is based on shrinking the point spreading function (PSF) engineering of the imaging system [1,2] by scanning the excitation spot. These techniques include confocal scanning microscopy, stimulated emission depletion microscopy (STED) [3], time-gated STED [4], ground-state depletion microscopy (GSD) [5], and fluorescence emission difference (FED) microscopy [6]. The other type is based on wide-field imaging, thereby omitting the complex configurations of scanning. The common principle of wide-field, super-resolution imaging is to impose some changes to the sample; at this stage, raw microcopy images carry partial spatial and temporal information of the sample, and thus a reconstruction process is necessary to obtain the super-resolution results. For instance, stochastic optical reconstruction microscopy (STORM) [7] and photo-activated localization microscopy (PALM) [8] rely on attaching special fluorochromes onto the samples, which results in the flashing of discrete molecules in response to different wavelengths. On the other hand, structured illumination microscopy (SIM) [9] projects a series of excitation light patterns on the sample, which cause normally inaccessible high-resolution information to be encoded in the observed image. As a wide-field optical microscopy technique, SIM has widespread applications for the observation of scattering or fluorescence samples. In general, all super-resolution methods require more than one low-resolution detection in order to recover the super-resolution information.

Fourier ptychographic microscopy (FPM) [10,11] is a newly developed super-resolution technique that employs time-sharing, multi-angle illumination and a phase retrieval algorithm to surpass the diffraction limit of the objective lens. As a result, FPM is able to obtain images with a large field-of-view (FOV) and wide space-bandwidth, without involving mechanical scanning or phase measurements. Moreover, FPM can be applied for scattering or refractive samples; that is because when samples are illuminated by light from a certain angle, a passive frequency shift occurs and the high-spatial-frequency information can be shifted to the propagation mode. However, FPM cannot be applied for fluorescence sample observation because the oblique illumination of different angles cannot change the propagating fluorescent field.

Against this background, we propose a Fourier ptychography approach using random illumination patterns to increase the resolution for fluorescence samples. However, it is difficult to exactly correspond the extra-high-frequency information involved in the result to the sample [12]. Thus the key technical problem for

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implementing FPM for fluorescence microscopy is to obtain the angular illumination images for the fluorescence samples, which inherit the wave vector of the illumination light. Fortunately, the angular illumination images can be extracted from the SIM images. That is because SIM projects a grating pattern on the sample, and the illumination pattern can be regarded as a superposition of one vertical and two symmetrical oblique coherent beams. This shifts the additional components of the fluorescence object spectrum into the microscope"s optical transfer function (OTF) domain through a frequency-mixing process [13]. Accordingly, we propose a new fluorescent imaging method, which we refer to as structured illumination fluorescence Fourier ptychographic microscopy (SIF-FPM). According to this method, the oblique illuminating images for the fluorescence sample are first extracted from the SIM images. Then, a novel optimized Fourier ptychographic algorithm is employed to reconstruct the super-resolution fluorescent results from the extracted oblique illumination images.

2. Method and algorithm

2.1. Oblique illumination image calculation

We demonstrated our method using a Nikon N-SIM microscope (numerical aperture (NA)=1.49, 100× oil) to acquire the original images. The process is shown in Fig. 1. The sample was imaged under a grating pattern illumination, which had a period of 250 nm. The vertical direction of the grating was set at 0°, 120°, and 240°; for each angle, three images were recorded when the phase of grating was set to 0, 2π/3, and 4π/3. Thus nine images were recorded during the acquisition process, with grating patterns that included different directions and phases.

At this stage, we extracted the equivalent oblique illuminated images of fluorescent samples from the SIM images, which stemmed from the conventional SIM algorithm. Imposing the grating pattern of a certain direction on the sample served as a lateral modulation for the sample"s intensity, which appeared as an additional term in the imaging process. The intensity distribution in the image plane recorded under structured illumination can be described as follows [14]:

$$I(r,\varphi)=\int O(r')[1+\sin(2\pi kr'+\varphi)]S(r;r')dr', \tag{1}$$

where r and r' represent the coordinates in the image plane and the analyzed sample plane, respectively, assuming that the magnification of the imaging system is 1. Further, k and φ denote the spatial frequency and the phase of the grating pattern, and $[1+\cos(2\pi kr'+\varphi)]$ is the pattern distribution of the illumination light. $O(r')$ is the spatial information of the analyzed sample, and $S(r;r')$ is the point spread function. Using Euler"s formula, the image can also be described as the sum of three components:

$$I(r,\varphi)=I'(r)+\frac{1}{2}e^{i\varphi}I''(r)+\frac{1}{2}e^{-i\varphi}I'''(r), \tag{2}$$

where the following equations hold:

$$I'(r)=\int O(r')S(r;r')dr' \tag{3a}$$

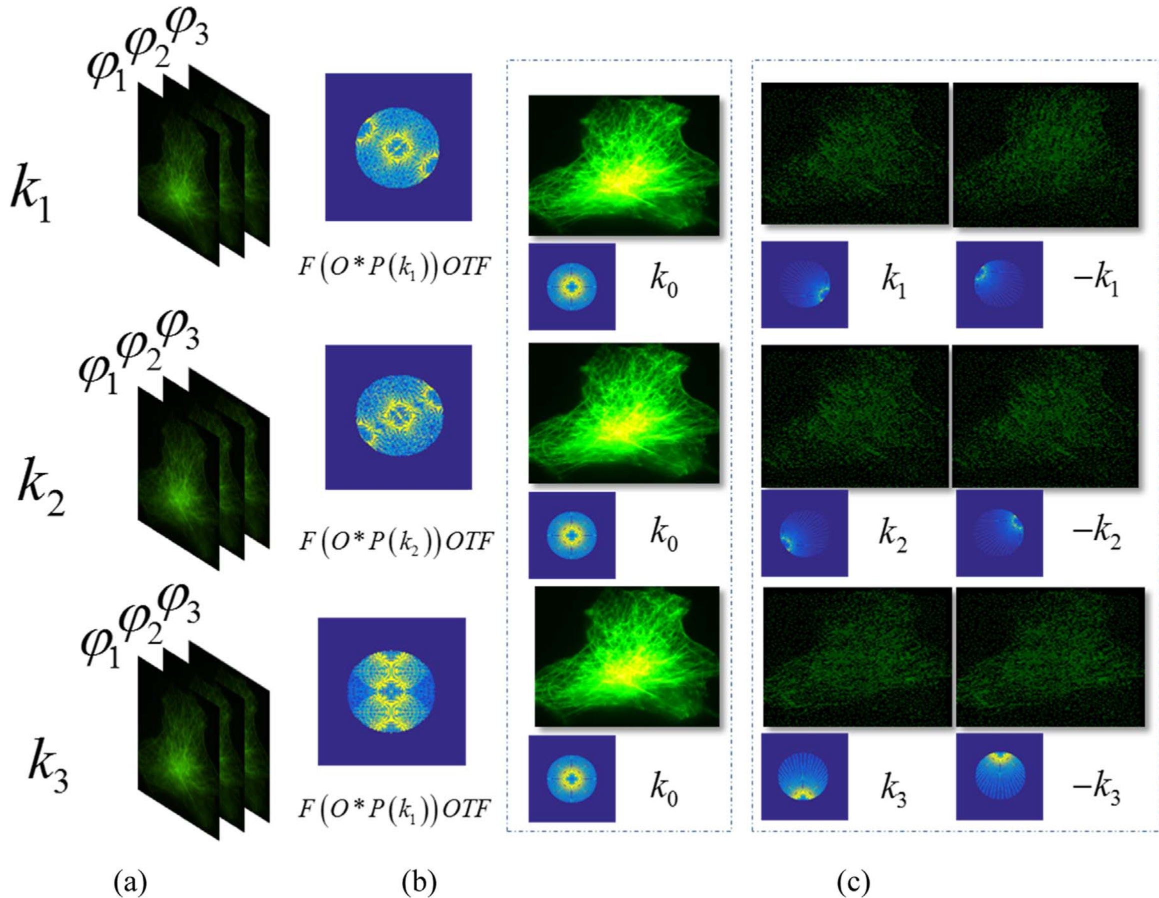

Fig. 1. Equivalent oblique illumination image calculation. (a) Nine images are recorded by structured illumination microscopy (SIM). k_1, k_2, and k_3 are the lateral vectors of the grating pattern; ϕ_1, ϕ_2, and ϕ_3 are the phases of the grating pattern. (b) The mixed Fourier domain in the aperture. (c) The calculated oblique illumination images with lateral wave vectors k_0, $\pm k_1$, $\pm k_2$, and $\pm k_3$.

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