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Emission difference super-resolution microscopy with optical lattices scanning and wide field detection

Dazhao Zhu [a], Youhua Chen [a,b], Cuifang Kuang [a,*], Xu Liu [a]

[a] State Key Laboratory of Modern Optical Instrumentation, College of Optical Science and Engineering, Zhejiang University, Hangzhou 310027, China
[b] Key Laboratory of Instrumentation Science & Dynamic Measurement of Ministry of Education, North University of China, Taiyuan 030051, China

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ABSTRACT

A parallel scanning method using optical lattices is proposed theoretically to improve the imaging speed of fluorescence emission difference microscopy (FED), which gives the wide field imaging capability to FED while maintaining all the basic advantages of single point FED. The basic principle of wide field FED (wfFED) is presented briefly and the method of generating optical lattices is discussed. The resolution via two types of optical lattices pattern scanning is also studied. With optical lattices scanning, which is generated by two orthogonally crossed standing waves, the wfFED can be implemented without wide field excitation. This strategy can further improve the wfFED imaging speed and simplify the set-up.

1. Introduction

Since the concept of super-resolution was proposed about twenty years ago, many methods have been invented and developed to break the diffraction barrier such as Photo Activated Localization Microscopy (PALM) [1], Stimulated Emission Depletion microscopy (STED) [2], Stochastic Optical Reconstruction Microscopy (STORM) [3], Structured Illumination Microscopy (SIM) [4], Saturated Structured Illumination Microscopy(SSIM) [5,6], Fluorescence Emission Difference microscopy(FED) [7–9], Super Resolution Optical Fluctuation Imaging (SOFI) [10] and so on.

As the development of modern science and technology, higher resolution is no longer the only goal, especially in biological imaging. The speed of imaging has gain more attention in recent years because researchers need to observe and study samples in very short time with a certain resolution in many situations, all of the super resolution methods mentioned above can provide high resolution, however, their imaging speed cannot meet the requirement of live-cell investigation. STED provides relatively high imaging speed, but it is still limited by fluorescent dyes [2]. Although FED does not suffer from this limitation, however, since the high requirement on scanning system to keep the quality of doughnut light, a scanning plate is generally used instead of scanning mirror. There are two main problems of FED, one is that the field of view is relatively small and the other one is the low imaging speed. In order to improve the imaging speed, we propose a wfFED with parallel scanning. Actually, several concept such as "parallelized microscopy" has been put up several years ago and many researchers and groups have been working on it since then [11–13].

Parallelized fluorescence microscopy was first demonstrated as SSIM [6,14]. Actually, SSIM is intrinsically parallelized for wide-field illumination and cameras for detection [4,5,15,16], and so as super resolution microscopy based on single molecule localization [3,17]. Unfortunately the imaging speed of SSIM is still limited because of the sophisticated data post-processing and accumulating frames requires long integration time. Super resolution microscopes based on single molecule localization wasted time on image reconstruction for the accumulation of a large number of frames [11]. The first parallelized configuration used in STED was reported many years ago, which utilized four pairs of scanning excitation and doughnut beams [11,12]. Lately a group uses optical lattices to realize massive parallelization of STED and another group realizes microscopy with more than 100,000 'doughnuts' [14].

* Corresponding author.
E-mail address: cfkuang@zju.edu.cn (C. Kuang).

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All these methods use multifocal spots array or optical lattices to realize parallel scanning and thereby improving the imaging speed. Therefore the quality of the spots array or optical lattices is the key factor of parallelized microscopy [18].

In this paper, we first use wide field illumination and optical lattices scanning, which can improve the imaging speed many times than single point FED, and then two types of optical lattices are also studied and analyzed. At last, the optical lattices generated by orthogonally standing waves is able to implement wfFED without wide field excitation. The zero areas in the optical lattices work as the central zero area in hollow spot, thus the whole imaging speed can be improved.

2. Theory

Fluorescence Emission Difference (FED) microscopy was proposed and realized experimentally in 2013 [7], which achieved a spatial resolution of $l/4\,\lambda$. The Fluorescence Emission Difference microscopy (FED) uses two beams—solid beam and hollow (doughnut) beam like that used in STED, to scan the sample respectively. The difference is that in STED the solid beam simulate fluorescence and the hollow beam is used for depletion, while in FED, both of the beams are used for exciting fluorescence. The two scanning beams obtain two images and then subtract hollow scanning image from solid scanning image, thereby a super-resolution image is soon reconstructed simply. This process can be described as:

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

where I_{solid} and I_{hollow} denote intensity of confocal images illuminated by the solid spot and hollow spot respectively, which have been normalized and r is the subtractive factor.

Compared to STED, the power of the laser being used in FED is lower. However the imaging speed of FED is much slower due to single point scanning and it wastes too much time on switching between solid spot excitation and hollow spot excitation. In order to improve the imaging speed of FED, wide field illumination and optical lattices scanning are applied, so that the process of switching between solid beam and hollow beam is omitted and transform the single point scanning to parallel scanning. The corresponding formula is:

$$I_{wFED} = I_{wf} - r \times I_{lattices} \tag{2}$$

where I_{wf} and $I_{lattices}$ denote normalized intensity of images illuminated by the wide field and optical lattices respectively and r is still the subtractive factor.

3. Simulation and discussions

Considering the basic strategy of wfFED, there is no pinhole to shrink the detection PSF, and in this case the resolution will be lower. So in order to study the resolution via optical lattices scanning and wide field detection, we first increase the number of illuminating hollow spots (doughnut) to four. As shown in Fig. 1(a), the four hollow spots have the same intensity distribution as the excitation hollow spot used in single point FED. When using these four spots to illuminate the sample, the simulated result is shown in Fig. 1(c). We set the wavelength of excitation light 488 nm, and the numerical aperture NA=1.42. According to the PSF we calculate, 1 pixel=9.5 nm, the wide field resolution according to Rayleigh Criterion is $\varepsilon = 0.61\lambda/\mathrm{NA} \approx 209.6$ nm ≈ 22 pixels. The sample (Fig. 1(b)) is a point array with 18 pixels (<22 pixels) distance between two points. Fig. 1(d) and (e) is the wide field

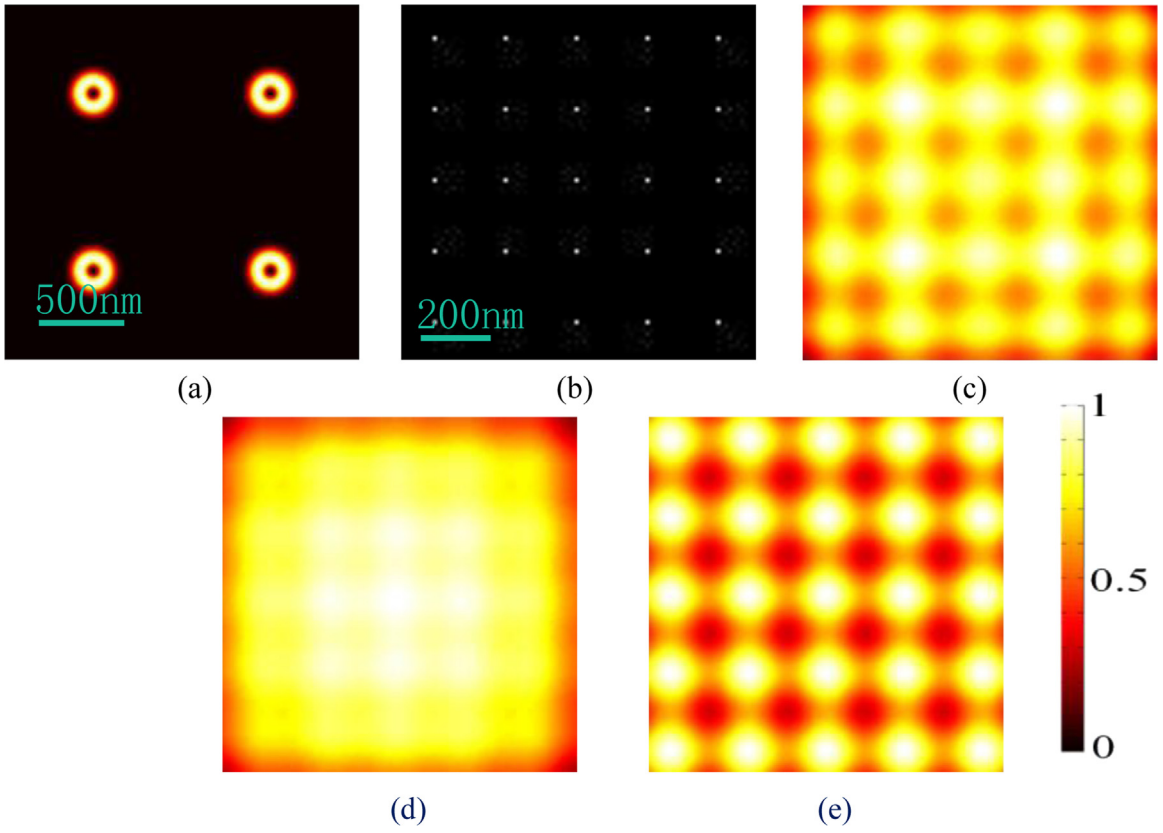

Fig. 1. (a) Optical lattices including four hollow spots. (b) Simulated sample. (c) wfFED image using (a) as illuminating light. (d) Wide field result (e) confocal result with 0.5 AU pinhole. Simulation result images are 200 pixels × 200 pixels, only central parts of the result images are shown to make the detail clear.

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