Temporal focusing microscopy with structured illumination for super-resolution deep imaging

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Abstract: We demonstrate that temporal focusing microscopy with structured illumination provides super-resolution even if wavefront distortion within the sample results in stretching the point spread function of the microscope.

OCIS codes: (180.4315) Nonlinear microscopy; (190.4180) Multiphoton processes.

1. Introduction

Temporal focusing (TF) microscopy provides the optical sectioning capability in wide-field two-photon excited fluorescence (TPEF) imaging [1, 2]. TPEF video-rate imaging over large areas of 5000-20000 μm^2 has been achieved by TF microscopy using amplified pulses [3, 4]. In a wide-field TPEF microscope, it is necessary to use a two-dimensional (2D) detector. Then, the fluorescence photons generated in a 2D spatial matrix within the sample must be imaged to their conjugate positions on the 2D detector. However, if the emitted photons are scattered within the sample, the scattered photons, which are not correctly mapped to the conjugate image position, turn into the background fluorescence in the image. In addition, the spatial resolution is degraded in deep imaging because of wavefront distortion within biological tissues [5]. Recently, the structured illumination technique has been used to reject the scattered background fluorescence and the out-of-focus background fluorescence in TF microscopy [6]. We have applied the structured illumination technique not only to reject the background fluorescence but also to enhance the lateral resolution [7]. Here, we demonstrate that interferometric TF (ITF) microscopy in which the structured illumination technique is combined with TF microscopy has a resistance to the resolution degradation due to wavefront distortion.

2. Interferometric temporal focusing microscopy

In wide-field fluorescence microscopy, the measured image can be described as a convolution of the emitted fluorescence with the point spread function (PSF) of the incoherent imaging system, $h(\mathbf{r})$. The TPEF image of samples acquired by an excitation light with a periodic pattern along the y direction can be expressed as [7]

$$S_m(\mathbf{r}) = \left[C(\mathbf{r})\left[I_0\left\{1+\alpha\cos(k_0 y+\phi_m)\right\}\right]^2\right]\otimes h(\mathbf{r}), \tag{1}$$

where $C(\mathbf{r})$ is the local fluorophore concentration, $\otimes$ denotes convolution, I_0 is the time-averaged intensity, α denotes a modulation depth, and k_0 and ϕ_m are the fundamental spatial frequency and phase shift of the periodic pattern, respectively. In Eq. (1), five spatial frequency components, $-2k_0$, $-k_0$, 0 (DC), $+k_0$, and $+2k_0$, are included in the periodic pattern. Through illumination with periodic patterns, high spatial frequency sample information is down-converted to a lower frequency, which can be supported by the optical transfer function (OTF) of the microscope. To extract each spatial frequency component by using the homodyne detection, we take five images, $S_m(\mathbf{r})$ at the phase shifts, $\phi_m=\phi_0+m\phi_s$, from m=0 to 4. Here the phase step, ϕ_s is $2\pi/5$, and ϕ_0 is the offset phase. After shifting the down-converted components back to their true position in Fourier space, the extracted spatial frequency components are recombined. The recombined image is given by [7]

$$F_{\mathrm{SR\_ITF}}(\mathbf{r}) = I_0^2 C(\mathbf{r})\otimes\left[h(\mathbf{r})\left\{a_0+a_1\cos(k_0 y)+a_2\cos(2k_0 y)\right\}\right], \tag{2}$$

where a_i is the weighting factor. We call the image reconstructed by Eq. (2) the super-resolution ITF (SR-ITF) image. Since the scattered background and the out-of-focus background do not produce the periodic pattern, they are contained in the DC component. Thus, in order to reject the background fluorescence, a_0 can be set to 0. If the wavefront is distorted within the sample, $h(\mathbf{r})$ stretches. However, $\cos(k_0 y)$ and $\cos(2k_0 y)$ do not change. Therefore, even if the wavefront is distorted, the lateral resolution is maintained at the width of $\cos(k_0 y)$ and $\cos(2k_0 y)$.

The non-super-resolution ITF (NSR-ITF) image, which is used only to suppress the background fluorescence, is reconstructed by [7]

$$F_{\mathrm{ITF}}(\mathbf{r}) = \left\{ \sum_{m=1}^{4} \left(S_0 - S_m\right)^2 + \sum_{m=2}^{4} \left(S_1 - S_m\right)^2 + \sum_{m=3}^{4} \left(S_2 - S_m\right)^2 + \left(S_3 - S_4\right)^2 \right\}^{1/2}. \qquad (3)$$

3. Experimental

The ITF microscope was constructed by combining a reflective beam splitter with the TF microscope using an optical parametric oscillator at 1064 nm [7]. We obtained TPEF images of an orange (540/560) fluorescent bead with a diameter of 200 nm to determine the spatial resolution of ITF microscopy with and without scattering samples. The scattering samples consisted of distillated water containing 2-μm polystyrene beads at concentrations of 2.9×10^8, 5.9×10^8, 8.8×10^8 beads/ml. Figure 1(a) shows the measured signal distribution of a 200-nm fluorescent bead along the lateral (y) direction with and without the scattering sample. The full width at half maximum (FWHM) for NSR-ITF was stretched by a factor of 1.5 due to wavefront distortion by the scattering sample, while the FWHM for SR-ITF was maintained. Figure 1(b) illustrates the dependence of the FWHM of the signal distribution on the beads concentration of the scattering sample. As increasing the beads concentration, the FWHM for NSR-ITF microscopy increased due to wavefront distortion. In contrast, the FWHM for SR-ITF was maintained around at 0.17 μm, which overcame the diffraction limit. Therefore, we found that SR-ITF has a resistance to the resolution degradation due to wavefront distortion.

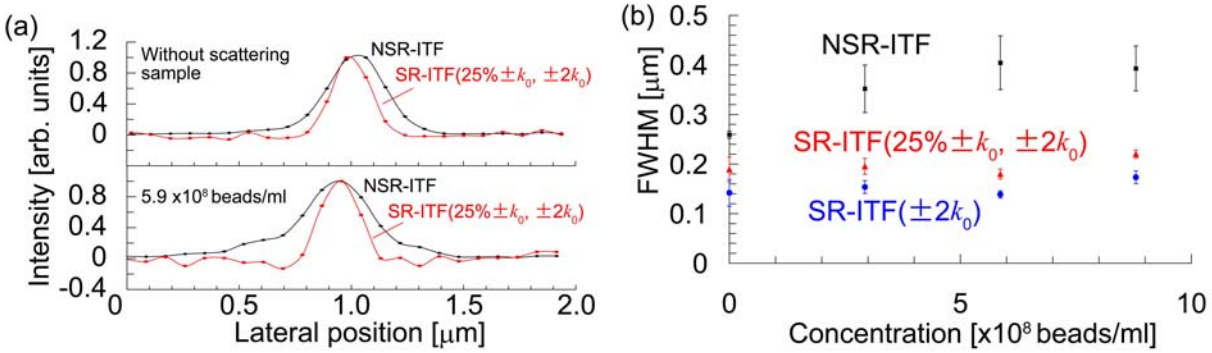

Fig. 1. (a) 1D signal distribution of a 200-nm fluorescent bead along the lateral (y) direction with (bottom) and without (top) the scattering sample, which contained polystyrene beads with a diameter of 2 μm at a concentration of 5.9×10^8 beads/mL. (b) FWHM of 1D signal distribution of a 200-nm fluorescent bead along the lateral (y) direction with the scattering sample at various concentrations.

4. Summary

We have demonstrated that the lateral resolution of SR-ITF can be maintained at less than diffraction limit even if the wavefront is distorted. SR-ITF would open up super-resolution imaging at deeper penetration.

5. References

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