Numerical study of super-resolved optical microscopy with partly staggered beams

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Abstract

The resolving power of optical microscopy involving two or even more beams, such as pump–probe microscopy and nonlinear optical microscopy, can be enhanced both laterally and longitudinally with partly staggered beams. A numerical study of the new super-resolution imaging technology is performed with vector diffraction theory. The influence of polarization is discussed. A resolving power of sub-100 nm and sub-300 nm in the lateral and longitudinal directions, respectively, is achievable.

Keywords: microscopy, super-resolution, partly staggered beams, vector diffraction theory

(Some figures may appear in colour only in the online journal)

1. Introduction

Optical microscopy has developed very rapidly in recent decades, and fluorescence microscopy is among the most powerful technologies with the capability of super-resolution imaging of samples stained with fluorescent labels. Several well-established super-resolution fluorescence techniques are now being used in biological and materials research, such as stimulated emission depletion (STED) [1, 2], stochastic optical reconstruction (STORM) [3] and photoactivated localization microscopy (PALM) [4]. However, fluorescence microscopy still has some limitations [5]: (1) many tissues, chromophores and molecules in biological systems absorb light but do not have efficient fluorescence emission, e.g. collagen and hemoglobin; (2) many intracellular small molecules are intrinsically nonfluorescent and they are also too small to be labeled with fluorescent proteins or dyes, e.g. neurotransmitters and drugs. As an alternative, fluorescence-free or label-free microscopies have been developed, such as pump–probe microscopy (PPM) [6–8], photothermal microscopy (PTM) [9–11] and nonlinear optical microscopy (NOM) [12–15]. Among these, PTM is very attractive for robust detection of single molecules or single nanoparticles as it has extremely high sensitivity [9–11]. Recently, PPM has become one of important tools in biological, medical, chemical and materials research. It can be used to study ultrafast dynamics or processes in chemicals, nanostructures and biological tissues within the sub-micrometer scale [16–18]. PPM can even be applied in some special fields such as examination of historical works of art [19]. NOM is also widely used in biological research [12–15]. There are also some super-resolution technologies suitable for label-free microscopies, such as nonlinear PTM [20–22], super-resolved coherent anti-Stokes Raman scattering (CARS) microscopies [23, 24] and super-resolved four-wave mixing microscopy [25]. Each of these super-resolved technologies works well for only one imaging mechanism or one kind of microscopy. These...
technologies also have their own limitations; for example, nonlinear PTM has a much lower signal intensity and higher risk of sample damage than linear PTM [20–22]. Besides the signal intensity loss (typically > 90%), super-resolved CARS based on ground-state depletion also needs a high-intensity depletion beam [23] and the application of a third beam will introduce complexity to the experimental system [23]. Therefore, it is still worthwhile to develop other super-resolved technologies for label-free microscopy.

In this paper, we demonstrate a type of super-resolution technology for several types of fluorescence-free and label-free optical microscopies involving two or more beams; we call it optical microscopy with partly staggered beams (OM-PSB). A detailed numerical study of OM-PSB is performed with vector diffraction theory. The influence of pump and probe polarization is discussed, including linear, circular, radial and azimuthal polarizations. Compared with traditional optical microscopy, the greatest improvement in resolution achieved with OM-PSB is >45% in the lateral direction and >35% in the longitudinal direction, with pump and probe beams of ideal plane waves. The simulation in this paper can help us choose the optimum experimental parameters.

2. Principle and numerical models

The pump and probe beams are partly staggered in a lateral (or longitudinal) direction at the focal point of the objective, and as a result the area of interaction of the two beams and the sample or the effective PSF (point spread function) of PTM can be reduced if the pump and probe beam shapes are carefully chosen. The signal intensity in PPM and PTM is proportional to the change of the probe beam intensity \( \Delta I_{\text{probe}} \), while that in NOM involving two beams, such as sum frequency generation (SFG), is the intensity of a newly generated signal \( I_{\text{new}} \). Both \( \Delta I_{\text{probe}} \) and \( I_{\text{new}} \) are proportional to the product of the intensities of the two beams involved \( I_{\text{pump}} \) and \( I_{\text{probe}} \), i.e. \( \Delta I_{\text{probe}} \propto I_{\text{pump}} \times I_{\text{probe}} \), under the small signal condition [5, 6]. Since the signals of NOM, PPM and PTM have similar relationships with pump and probe intensities (similar effective PSFs), we only discuss the cases of PPM and PTM with partly staggered beams (PPM-PSB) in this paper.

Table 1. Polarization matrix unit for different polarizations [27, 28].

| Polarization | Polarization matrix unit |
|--------------|--------------------------|
| Linear       | [0, 0, 1]; [0; 1; 0]     |
| Left circular| [1; i; 0]/\( \sqrt{2} \) |
| Right circular| [1; –i; 0]/\( \sqrt{2} \) |
| Radial       | [\( \cos\phi \); \( \sin\phi \); 0] |
| Azimuthal    | [–\( \sin\phi \); \( \cos\phi \); 0] |

Debye integral [26–28] as follows:

\[
E_{x,y,z}(x, y, z) = \frac{iC}{\lambda} \int_0^{\alpha \arcsin(NA/n)} A_{\text{AMP}} \cdot A_{\text{Phase}} \cdot A_L \left[ \begin{array}{c} P_x \\ P_y \\ P_z \end{array} \right] \sin \theta \cdot e^{i(k_z z + \sqrt{x^2+y^2} \sin \theta \cos(\phi - \arctan(y/x)))} d\theta d\phi
\]

(1)

where \( \vec{E}(x, y, z) \) is the electric field vector, \( C \) is the normalization constant, \( A_{\text{AMP}} \) and \( A_{\text{Phase}} \) are the real amplitude and phase of the input light, respectively. \( A_{\text{AMP}} = 1 \) for idea plane wave, \( A_{\text{Phase}} = e^{i\phi} \) for an azimuthal polarized pump and probe, in this case, a 0–2\( \pi \) vortex phase plate is applied to modulate the beam shapes to achieve tightly focused solid focal spots rather than donut ones. \( A_{\text{Phase}} = 1 \) for linear and circular polarizations. \( A_L \) is the vector weight matrix of the objective lens. With an anaplastic lens as the focal lens, \( A_L \) can be expressed as follows [27, 28]:

\[
A_L(\theta, \phi) = \sqrt{\cos \theta} \cdot \begin{bmatrix} 1 + (\cos \theta - 1) \cos^2 \phi & (\cos \theta - 1) \cos \phi \sin \phi - \sin \theta \cos \phi \\ (\cos \theta - 1) \cos \phi \sin \phi - \sin \theta \cos \phi & 1 + (\cos \theta - 1) \sin^2 \phi - \sin \theta \sin \phi \end{bmatrix} \cdot \begin{bmatrix} \sin \theta \sin \phi \\ \cos \theta \end{bmatrix}.
\]

(2)

\( [P_x; P_y; P_z] \) is the polarization matrix unit for the polarizations of the input beams, and values for linear, left circular, right circular, radial and azimuthal beams are shown in Table 1.

The PSFs of pump and probe beams are calculated by \( I(x, y, z) = |E_x|^2 + |E_y|^2 + |E_z|^2 \). While the effective PSF of PPM-PSB is defined by \( \Delta I(x, y, z) = I_{\text{pump}}(x + \Delta x/2, y + \Delta y/2, z + \Delta z/2) I_{\text{probe}}(x-\Delta x/2, y-\Delta y/2, z - \Delta z/2) \), where \( I_{\text{pump}} \) and \( I_{\text{probe}} \) are intensity distributions of pump and probe beams and \( \Delta x, \Delta y \) and \( \Delta z \) are the offset of pump and probe beams in the \( x, y \) and \( z \) directions, respectively. To fit the common experimental conditions, the numerical aperture (NA) of the focal lens is assumed to be 1.4 (100\% oil immersion), and the wavelengths of pump and probe beams are set as 488 and 632 nm, respectively. By solving the above equations, PSFs of pump beam and probe beam normally used in pump–probe microscopy (i.e. no spatial offset between pump and probe) (NPPM) and PPM-PSB are obtained.
3. Results and discussions

For a qualitative demonstration of PPM-PSB, the effective PSFs of PPM-PSB are given together with the PSFs of the pump beams, probe beams and NPPM. With the offset of pump and probe only in the $x$ direction, the PSFs of pump, probe, NPPM and PPM-PSB in the $xy$ plane for different polarizations are shown in figure 1. The first column in figure 1 is the PSFs of pump (blue spots) and probe (red spots) beams, while the second and third columns are the effective PSFs of NPPM and PPM-PSB, respectively. In the case of PPM-PSB, the values of the offset $\Delta x$ are 350, 225, 290, 520 and 150 nm for $L_x$, $L_y$, $C_r$, $R_a$ and $A_z$ polarizations, respectively.

![Figure 1](image1.png)

Figure 1. Effective PSFs of pump (blue spots in the first column), probe (red spots in the first column), NPPM (bright spots in the second column) and PPM-PSB (bright spots in the third column) in the $xy$ plane with different polarizations. $L_x$, linear polarization with the direction of polarization along the $x$-axis; $L_y$, linear polarization with the direction of polarization along the $y$-axis; $C_r$, right circular polarization; $R_a$, radial polarization; $A_z$, azimuthal polarization. The values of $\Delta x$ are 350, 225, 290, 520 and 150 nm for $L_x$, $L_y$, $C_r$, $R_a$ and $A_z$ polarizations, respectively.

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Figure 2 shows the axial distribution of the effective PSFs for PPM-PSB. The offset of pump and probe is set only in the $z$ direction with $\Delta z$ of 610, 615, 700 and 620 nm for $L_x$, $C_r$, $R_a$ and $A_z$ polarizations, respectively. With the intensities of the side lobes kept smaller than 25% of the main peak intensity for all cases. The dynamic ranges of the figures for PPM-PSB are also different from that for NPPM. As a result, the size of PPM-PSB in the $x$ direction seems to be even larger than that of NPPM for all four polarizations. However,
if we take the cross sections of the PSFs in figure 2 for comparison, PPM-PSB and NPPM have similar PSF sizes in the x direction, and the former has a smaller PSF size in the z direction. The sizes of the cross sections in the x and z directions of the PSFs for PPM-PSB will also be given later.

A quantitative discussion of PPM-PSB will be given below. We have calculated the sizes of the effective PSFs for different offsets ($\Delta x$ or $\Delta z$) and polarizations (parts (a) and (b) in figures 3–9). To reduce the calculation time to an acceptable range, one step of the offsets ($\Delta x$ or $\Delta z$) in the calculation is set to be 5 nm. As a result, pixellation-like noise appears in the results, as shown in parts (a) of figures 3–9. This kind of noise will not greatly influence the demonstration of the method. Since the offsets of pump and probe reduce the peak intensity of the effective PSFs, bringing large side lobes, the relationship between peak intensity reduction and the offsets is also studied for different pump and probe polarizations (parts (c) in figures 3–9). In the calculation, the maximum offset of pump and probe is set taking into account of the sizes of the side lobes, the intensity of which is kept below 25% of the intensity of the main peak. The intensity profiles of the effective PSFs of PPM-PSB with the maximum offset are also shown (parts (d) in figures 3–9). Figures 3–5 demonstrate the characteristics of the lateral components of the effective PSFs for PPM-PSB with different pump and probe polarizations. Figure 3 shows the case of Lx polarized pump and probe beams. The full width at half maximum (FWHM) of the effective PSF of PPM-PSB reduces from 196 nm to 128 nm along the x-axis with $\Delta x$ increased from 0 to 350 nm (denoted by the blue curve in figure 3(a)), with an improvement in resolving power of $\sim34.7\%$ and $45.8\%$ from NPPM (blue curve in figure 3(a)) and conventional optical microscopy with the excitation of the pump beam (black curve in figure 3(a)), respectively. Figure 3(b) shows that the FWHM of the main peak of effective PSF size along the y-axis is not dependent on $\Delta x$ in the range of 0–350 nm. With linearly polarized pump and probe, the effective PSF of NPPM has different sizes in the x and y directions, as shown in figure 1 and the red curves in figures 3(a) and (b). The difference can be removed by utilizing PPM-PSB with carefully chosen $\Delta x$, i.e. the FWHM values of the effective PSF along x- and y-axes are both equal to 132 nm in the case of $\Delta x \sim 340$ nm. As shown in figure 3(c), the peak intensity decreases with increase in the offset $\Delta x$. With $\Delta x = 350$ nm, the peak intensity in the PPM-PSB mode is about 10% of that with the NPPM mode. Figure 3(d) shows the intensity profiles of the effective PSF of

Figure 3. For Lx polarization, the dependence of the FWHM of effective PSFs along the x- (a) and y-axis (b) on $\Delta x$. (c) The dependence of the peak intensity of effective PSF for PPM-PSB on $\Delta x$. (d) The intensity distributions of PPM-PSB along the x- and y-axis for $\Delta x = 350$ nm.
Figure 4. For Ly polarization, the dependence of the FWHM of effective PSFs along the x- (a) and y-axis (b) on Δx. (c) The decrease in peak intensity of the effective PSF of PPM-PSB with increasing Δx. (d) The intensity distributions of PPM-PSB along the x- and y-axis for Δx = 237 nm.

Figure 5. For Cr polarization, the dependence of the FWHM of effective PSFs along the x- (a) and y-axis (b) on Δx. (c) The peak intensity of the effective PSF of PPM-PSB decreases with increasing Δx. (d) The intensity distributions of PPM-PSB along the x- and y-axis for Δx = 290 nm.
Figure 6. For Lx polarization, the dependence of the FWHM of the effective PSFs along the x- (a) and z-axis (b) on Δz. (c) The peak intensity of the effective PSF of PPM-PSB decreases with increasing Δz. (d) The intensity distributions of PPM-PSB along the x- and y-axis for Δz = 610 nm.

Figure 7. For Cr polarization, the dependence of the FWHM of the effective PSFs along the x- (a) and z-axis (b) on Δz. (c) The peak intensity of the effective PSF of PPM-PSB decreases with increasing Δz. (d) The intensity distributions of PPM-PSB along the x- and y-axis for Δz = 615 nm.
PPM-PSB mode along both \( x \) - and \( y \)-axes. The side lobe intensity is about 22\% of the peak intensity, and the peak position of the effective PSF of PPM-PSB also shifts by 36 nm along the \( x \)-axis with \( \Delta x = 350 \) nm. The cases for Ly and Cr polarized pump and probe are shown in figures 4 and 5, and the detailed information is summarized in table 2. From figure 4(a) and table 2, we can find that the minimum size of the effective PSF along the \( x \)-axis for PPM-PSB can reach 100 nm for a Ly polarized pump and probe. The resolving power improvement for PPM-PSB is \( \sim 28.5\% \) and 41.9\% compared with NPPM and conventional optical microscopy with excitation of the pump beam, respectively. Typically, the peak intensity is \( \sim 10\% \) of the maximum value with side lobes of \( \sim 20\% \) of the main peak, as shown in parts (c) and (d) of figures 3–5 and table 2. The cases for Ra and Az polarized pump and probe are not given here, for nearly no improvement in resolving power can be observed for PPM-PSB with these two types of polarization even with \( \Delta x \) optimized, as shown in figure 1.

Figures 6–9 show the characteristics of the longitudinal components of the effective PSFs for PPM-PSB with Lx, Cr, Ra and Az polarized pump and probe, respectively. A summarization of the calculated data is shown in table 3. From figures 6–9 and table 3, we can see that the typical size of the effective PSF along the \( z \)-axis is \( \sim 300 \) nm for PPM-PSB, with an improvement in resolving power of 20\%–30\% and 30\%–40\% compared with NPPM and conventional optical microscopy with excitation of the pump beam, respectively. The reduction in peak intensity is typically \( \sim 85\% \) with the side lobes smaller than 25\% of the main peak. The size of the PSF in the \( x \) direction will become worse with increased \( \Delta z \) for Lx and Cr polarized beams, as shown in figures 6(b) and 7(b). The minimum size of the effective PSF along the \( z \)-axis can reach 288 nm with an Az polarized pump and probe, and the improvement in resolving power is \( \sim 24.2\% \) compared with NPPM.

Similar to most types of super-resolution microscopy, the main drawback of the imaging technology reported here using a partly staggered pump and probe is also the reduction in peak intensity with the increased offset (resolution). One trivial solution for this problem is just to increase in the pump and probe powers. The applications of a detection system with higher sensitivity and lower noise, such as a lock-in detection system or a single photon counter, also will help to maintain a high enough signal to noise ratio (SNR) for the imaging. The relatively large (typically \( \geq 10\% \)) side lobes will also introduce some distortions to the imaging. The problems of limited enhancement of the
resolving power and large side lobes may be solved by applying other beam shapes for the pump and probe. Further work about the influence of beam shapes is still necessary and in progress.

The overlapping of the two beams can be measured and monitored by a backscattering mode of the microscope with a standard sample, such as a sample of gold nanoparticles or a metal film, as described in our previous paper [22]. The lateral offset of pump and probe can be adjusted with the deflection angle of the beam coupler for the two beams, while the longitudinal offset can be adjusted by tuning the divergence angle of the pump or probe [30].

Figure 9. For Az polarization, the dependence of the FWHM of the effective PSFs along the x- (a) and z-axis (b) on Δz. (c) The peak intensity of the effective PSF of PPM-PSB decreases with increasing Δz. (d) The intensity distributions of PPM-PSB along the x- and y-axis for Δz = 620 nm.

Table 2. Lateral properties of PPM-PSB with the offset of pump and probe along the x-axis. \( I_{p0} \) and \( I_{p} \) are the peak intensities of the PSFs of NPPM and PPM-PSB, respectively. \( I_{s} \) is the intensity of the side lobes.

| Polarization | \( \Delta x \) (nm) | Pump x (nm) | NPPM x (nm) | PPM-PSB x (nm) | \( I_{p0}/I_{p} \) | \( I_{s}/I_{p} \) |
|--------------|-----------------|-------------|-------------|----------------|----------------|----------------|
| Lx           | 350             | 236         | 196         | 128            | 9.3%           | 22.2%          |
| Ly           | 237             | 172         | 140         | 100            | 12.1%          | 24.9%          |
| Cr           | 290             | 204         | 164         | 140            | 10.2%          | 19%            |

Table 3. Longitudinal properties of PPM-PSB with the offset of pump and probe along the z-axis. \( I_{p0} \) and \( I_{p} \) are the peak intensities of the PSFs of NPPM and PPM-PSB, respectively. \( I_{s} \) is the intensity of the side lobes.

| Polarization | \( \Delta z \) (nm) | Pump z (nm) | NPPM z (nm) | PPM-PSB z (nm) | \( I_{p0}/I_{p} \) | \( I_{s}/I_{p} \) |
|--------------|-----------------|-------------|-------------|----------------|----------------|----------------|
| Lx           | 610             | 484         | 388         | 308            | 18.3%          | 23.9%          |
| Cr           | 615             | 484         | 388         | 308            | 17.9%          | 24.6%          |
| Ra           | 700             | 508         | 412         | 308            | 13.5%          | 20.9%          |
| Az           | 620             | 476         | 380         | 288            | 15.9%          | 26%            |
In the above, we discuss the improvement in resolving power of PPM-PSB in lateral and longitudinal directions separately. Actually, the improvement in resolving power can be obtained simultaneously in lateral and longitudinal directions with the offset of pump and probe along both x- and z-axes. As a result, high-resolution, three-dimensional imaging can be achieved. However, the signal reduction will be even larger, and it may give a low SNR to the image.

The super-resolution imaging technology demonstrated in the present paper is also compatible with other high-resolution microscopies, such as nonlinear PTM (NLPTM) [20–22] and PPM with an annular pupil [31]. By combining such technologies an even higher resolving power is achievable with multiplex super-resolution imaging technologies, for example NLPTM with partly staggered beams (NLPTM-PSB).

4. Summary

In conclusion, a super-resolution imaging technique for several types of widely used, fluorescence-free and label-free optical microscopies, i.e. PPM, PTM and NOM, is demonstrated. A detailed simulation is performed using vector diffraction theory. The simulation shows that sub-100 nm resolution in the lateral direction and sub-300 nm in the longitudinal direction is achievable by this method, with pump and probe wavelengths of 488 and 632 nm, respectively. The improvement in lateral resolving power for OM-PSB is typically ~30% and 40% compared with NPPM and traditional optical microscopy, respectively. The resolving power of OM-PSB in the longitudinal direction can be improved by typically ~20% and 30% compared with NPPM and traditional optical microscopy, respectively. The influence of polarization is discussed. The highest resolving power in the lateral direction is achieved with an Ly polarized pump and probe, while that in longitudinal direction is obtained with Az polarized beams.

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