Parallel multiphoton microscopy with cylindrically polarized multifocal arrays

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Diffraction-limited cylindrically polarized multifocal arrays are created in the focal region of a high numerical-aperture objective for multiphoton microscopy by applying the dynamic phase modulation on an incident light beam. We show that this kind of cylindrical-polarization multifocal multiphoton microscopy exhibits a parallel imaging capacity but also a dynamic switching-on or -off feature of individual focal spots. The parallel multiphoton microscopy results of the polarization-sensitive gold nanorods under the illumination of radially or azimuthally polarized multifocal arrays allow for the fast determination of the orientation of nanorods. © 2013 Optical Society of America

Cylindrically polarized laser beams, including radially polarized beams and azimuthally polarized beams, have attracted intensive attention in optical microscopy [1], laser tweezing [2], optical data storage [3], plasmonic superlensing [4], and direct laser writing [5] because they provide a rich polarization feature in the focal region. In particular, multiphoton optical microscopy under radial polarization illumination has allowed for the enhancement of photo thermal therapy [6], the determination of the molecular ordering in lipids [7], and the 3D orientation of nanorods [8]. To increase the frame rate and laser power efficiency in the imaging process, many researchers have developed multifocal multiphoton microscopy (MMM) by using different optical geometries, including microlens arrays [9], beam splitters [10], etalons [11], and diffractive optical elements [12]. However, these approaches are not physically feasible to convert a cylindrically polarized beam into a multifocal array with identical cylindrically polarized foci.

On the other hand, a spatial light modulator (SLM) has been widely used for wavefront shaping [13–16], including the generation of linearly polarized multifocal arrays [13,15]. In the presence of a high numerical-aperture (NA) objective, however, it has been impossible to produce a cylindrically polarized multifocal array through direct wavefront shaping. In this Letter, for the first time we show the experimental generation of the cylindrically polarized multifocal arrays with a controllable identical polarization state at each focal spot through the phase modulation on the incident light beam focused by a high NA objective. By implementing the generated multifocal arrays with an SLM [Fig. 1(a)], a cylindrical-polarization MMM setup can be developed for fast parallel imaging of fluorescent beads and polarization-sensitive gold nanorods [Figs. 1(b)–1(g)]. The parallel multiphoton microscopy (PMM) results of gold nanorods show not only the identical radial or azimuthal polarization state achieved at each focus in the array but also the orientation of the nanorods.

The generation of a cylindrically polarized multifocal array is based on the use of an accurate phase modulation function derived from the Debye diffraction-based iterative method [15–17]. For a given polarization state of an incident beam, a phase modulation function for generating a multifocal array in the focal plane of a high NA objective can be iteratively calculated through the Debye diffraction theory [17] and Fourier transform [15]. This phase function multiplied by the electric field distribution of the incident beam is then placed at the back aperture of the objective, which can finally lead to a

Fig. 1. (a) Experimental setup for PMM using an SLM. L, lenses; M, mirrors; PC, polarization converter; HPSF, high-pass spatial filter; BS, beam splitter cube; SPF, short-pass filter; O, objective lens (1.4 NA, 100×); S, sample; SS, scanning stage. (b)–(d) Generated phase modulation function for a 2 × 2 radially polarized multifocal array, the corresponding intensity distribution in the focal region, and the PMM image of fluorescent microspheres. (e)–(g) Generated phase modulation function for a 2 × 2 azimuthally polarized multifocal array, the corresponding intensity distribution in the focal region, and the PMM image of fluorescent microspheres. Insets: enlarged images of one of the focal spots in the radially and azimuthally polarized multifocal arrays (marked using white squares). Scale bar: 10 μm.
multifocal array with each focal spot having the polarization state identical to the incident beam [16]. As an example, the phase modulation function and the corresponding 2 × 2 multifocal arrays for radially and azimuthally polarized incident beam illumination are depicted in Figs. 1(b), 1(c), 1(e), and 1(f). As demonstrated, the generated 2 × 2 arrays are diffraction limited with an identical polarization state [16].

Figure 1(a) shows an experimental setup of a high NA PMM system using an SLM to create cylindrically multifocal arrays. A linearly polarized femtosecond pulsed laser beam (Spectra-Physics MaiTai, 100 fs, 80 MHz) working at the wavelength of 800 nm was illuminated on an SLM (Holoeye Pluto, 1080 × 1920 pixels, 256 gray levels) through the beam expansion system composed of lenses L1 (∼50 mm) and L2 (400 mm). The phase modulation generated by the SLM was transferred to the back aperture of a high NA objective (Olympus, 100×, UPLSAPO, 1.40 NA) through a 4f imaging system composed of lenses L3 (200 mm) and L4 (125 mm). A polarization converter (Arcopix) [18] was used to convert the linear polarization state into the cylindrical polarization state. A high-pass spatial filter was placed at the Fourier plane of lens L3 to block the zeroth order. The pulsed laser beam with the phase pattern was then focused by the high NA objective to produce radially and azimuthally polarized 2 × 2 multifocal arrays that had a high-intensity uniformity (99%) [15]. These arrays were used to induce multiphoton excited fluorescence from samples placed on a 3D piezo scanning stage. The fluorescence signal reflected from the nonpolarization beam splitter cube was focused by lens L5 (150 mm) and collected by an intensified CCD (ICCD) camera. A short-pass filter (<694 nm) was placed in front of the ICCD camera to suppress the reflected and scattered laser beams.

In order to achieve PMM, the ICCD camera was programmed to be divided into four (not limited to four) areas to collect the fluorescence signals from each of the four focal spots individually. An aperture with four holes (500 µm in diameter) was placed in front of the ICCD camera [the inset of Fig. 1(a)], so that the fluorescence signal from each focus in the array could be separately collected by the corresponding hole. Therefore, the complete image recorded with the ICCD camera from each of the four holes minimized the background noises and the cross talk between different holes. The distance between the holes D is defined as d × M, where d is the distance between the focal spots in the multifocal array (30 µm) and M is the magnification rate (83 in the study) of the imaging system. Therefore, D is equal to 2.5 mm in our case.

To illustrate the PMM capability, we used 10 µm fluorescent microspheres (FluoSpheres, peak fluorescence at the wavelength of 525 nm) as the sample. The parallel imaging of the fluorescent microspheres by using the 2 × 2 multifocal arrays via the two-photon excitation (TPE) process is given in Figs. 1(d) and 1(f). The field of view (FOV) of the image is 60 µm × 60 µm consisting of 1600 × 1600 pixels, in which each focal spot is scanned over an area of 30 µm × 30 µm at a speed of 100 µm/s. An overall input power measured at the back aperture of the objective is 4 mW. As the fluorescence intensity generated from the microspheres depends on the intensity of the focal spots, a higher excitation efficiency under the radially polarized beam illumination can be achieved than that under the azimuthally polarized beam illumination due to the stronger peak intensity of the focal spots at the same incident power in the former case [Figs. 1(d) and 1(g)]. Because the fluorescent microspheres are isotropic, these images show little difference between the two polarization illumination cases.

Dynamically manipulating the number of foci and the geometric arrangement of the multifocal arrays is shown in Figs. 2(a), 2(d), and 2(g) by updating the phase modulations displayed on the SLM. For each case we rearranged the focal spot geometry [Figs. 2(b), 2(e), and 2(h)] by turning off one or two of the focal spots. The dark regions in Figs. 2(c), 2(f), and 2(i) indicate that the focal spots are switched off.

For the demonstration of the polarization sensitivity of the PMM system we applied the generated multifocal arrays to image the polarization-sensitive gold nanorods. Gold nanorods with the longitudinal surface plasmon resonance at the wavelength of 790 nm were prepared by wet chemical methods [10] and added to polyvinyl alcohol (PVA) with a volume ratio of 1 : 1. Then a single layer of the PVA film containing the gold nanorods was spin coated on a coverslip [8]. As is well known [20], the TPE efficiency of the gold nanorods is maximized only when the longitudinal axes of the nanorods are aligned to the polarization direction in the focal region of the laser beam.

The PMM image of the gold nanorods by using a radially polarized multifocal array is shown in Fig. 3(a). The FOV of the image is set to be 40 µm × 40 µm and the excitation power is 1.2 mW to avoid photodamage of gold nanorods. It can be seen from Fig. 3(a) that

![Fig. 2.](image)

(a) Phase
(b) Focusing geometry
(c) Image
(d) (e) (h) Schematics of the focusing geometry corresponding to the phase modulation functions. (c), (f), and (i) Corresponding PMM images by using the multifocal arrays.
two-lobe-shaped patterns corresponding to the intensity
distribution of field components along the longitudinal
axes of the gold nanorods appear in the images collected
by each of the focal spots. A detailed comparison of a
single gold nanorod imaged with the multifocal spot ar-
ray and an unmodulated single radially polarized focal
spot is displaced in Figs. 3(b)–3(f), revealing an identical
two-lobe-shaped distribution. The two-lobe shape with a
local minimum line (marked by the white line at the
center of the two-lobe pattern) perpendicular to the
longitudinal direction of the nanorod (marked by the yel-
low double arrows) agrees well with the simulation result
[Fig. 3(g)]. This result indicates that the same radial
polarization state is achieved at each focus in the array.

PMM of gold nanorods with the azimuthally polarized
multifocal array is depicted in Fig. 4(a). With the azimu-
thally polarized multifocal array and an unmodulated sin-
gle azimuthally polarized focal spot, a single gold nanorod
is imaged [Figs. 4(b)–4(f)]. Unlike Figs. 3(b)–3(f), a two-
lobe shape with the local minimum line parallel to the
longitudinal axis of the nanorod appears, which shows
a good agreement with the simulation result [Fig. 4(g)].

In conclusion, we have demonstrated the experimental
generation of diffraction-limited cylindrically polarized
2 × 2 multifocal arrays by applying the phase modulation
onto an incident linearly polarized beam focused by a
high NA objective. The generated multifocal arrays have
facilitated a dynamic imaging system with an SLM for
PMM of fluorescent microspheres and gold nanorods.
The two-photon images of the gold nanorods show that
the identical radial or azimuthal polarization state and
the orientations of the nanorods can be determined at
each focus in the array. The work presented in this Letter
can open up a route to quickly determine 3D orientations
of single molecules and realize parallel second- and third-
harmonic generation in biology samples with the reduc-
tion of unwanted scattering [1,7]. Dynamic PMM is poten-
tially a new tool for parallel operation in optical tweezers
[2], optical data storage [3,8,20], high light-directing devi-
ces [21], and direct laser writing [22].

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References
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