2D simultaneous spatial and temporal focusing multiphoton microscopy for fast volume imaging with improved sectioning ability

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ABSTRACT

Simultaneous spatial and temporal focusing (SSTF) multiphoton microscopy offers us widefield imaging with sectioning ability. As extending the idea to 2D SSTF, people can utilize a 2D spectral disperser. In this study, we use a 2D spectral disperser via a virtually-imaged phased-array (VIPA) and a diffraction grating to fulfill the back aperture of objective lens with a spectrum matrix. This offers us an axial resolution enhanced by a factor of ~1.7 compared with conventional SSTF microscopy. Furthermore, the small free spectral range (FSR) of VIPA will reduce the temporal self-imaging effect around out-of-focus region and thus will reduce the out-of-focus multiphoton excited fluorescence (MPEF) signal of 2D SSTF microscopy. We experimentally show that inside a sample with dense MPEF, the contrast of the sectioning image is increased in our 2D SSTF microscope compared with SSTF microscope. In our microscope, we use a 1 kHz chirped amplification laser, a piezo stage and a sCMOS camera integrated with 2D SSTF to realize high speed volume imaging at a speed of 50 volumes per second as well as improved sectioning ability. Volume imaging of Brownian motions of fluorescent beads as small as 1μm has been demonstrated. Not only the lateral motion but also the axial motion could be traced.

Keywords: three-dimensional microscopy, nonlinear microscopy, wide-field microscopy

1. INTRODUCTION

For neuroscience, fast volume imaging is necessary. The common imaging tool is multi-photon laser-scanning microscopy (MPLSM). It uses a point detector with time-division multiplexing to acquire the information of each pixel. The scanner speed limits the frame rate. Recently in order to boost up the frame rate, simultaneous spatial and temporal focusing (SSTF) technique and light-sheet technique were invented [1-3]. They both utilize parallel detector such as CMOS camera to simultaneously illuminate and acquire the whole frame. Due to the fact that light-sheet microscopy need to illuminate from the side of the sample, it is hard to do in-vivo imaging such as living mouse brain imaging. So SSTF is an ideal method for neuroscience study.

Previously, the chirped pulse amplification (CPA) femtosecond laser was integrated for SSTF microscopy to achieve two-photon excitation fluorescence (TPEF) microscopy at 100 frames/sec with a field of view of 200 μm by 100 μm [4]. However, the axial confinement ability is limited as $I_{TPE}(z)\sim\left[1+(z/z_R)^2\right]^{-0.5}$ where $I_{TPE}(z)$ is the two-photon excitation (TPE) intensity and z_R is the Rayleigh length. So in order to improve it to be the same as MPLSM case, $I_{TPE}(z)\sim\left[1+(z/z_R)^2\right]^{-1}$, we proposed the 2D SSTF technique which utilizes a 2D spectral disperser with extreme small free spectra range (FSR). Not only the axial resolution is improved by a factor of ~1.7 but also the out-of-focus excitation intensity is suppressed [5].

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Multiphoton Microscopy in the Biomedical Sciences XV, edited by Ammasi Periasamy, Peter T. C. So, Karsten König,
Proc. of SPIE Vol. 9329, 93292N · © 2015 SPIE · CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2078093

2. METHOD AND SETUP

Figure 1 shows the setup of 2D SSTF microscopy. A 2D disperser combines a grating and a virtually imaged phased array (VIPA). The dispersing direction is orthogonal to each other. By using collimating optics, we get a dispersed matrix at Fourier plane. After that, an objective lens is used to collect all the spectrum components at the focal plane and we pre-compensate the pulse to be Fourier transform limited pulse at the focal plane. Compared to the SSTF which uses grating only, the out-of-focus pulse is further stretched in 2D SSTF. This further reduces the out-of-focus nonlinear excitation efficiency and thus improves the axial confinement ability. The light source is a CPA laser. The center wavelength is 803 nm and the pulse duration is~50 fs (FWHM) with a repetition rate of 1 kHz. In order not to damage the VIPA, we put the grating in front of the VIPA. By this setting, the pulse is first spatially chirped at the input window of the VIPA and thus we can boost up enough power for wide-filed deep imaging.

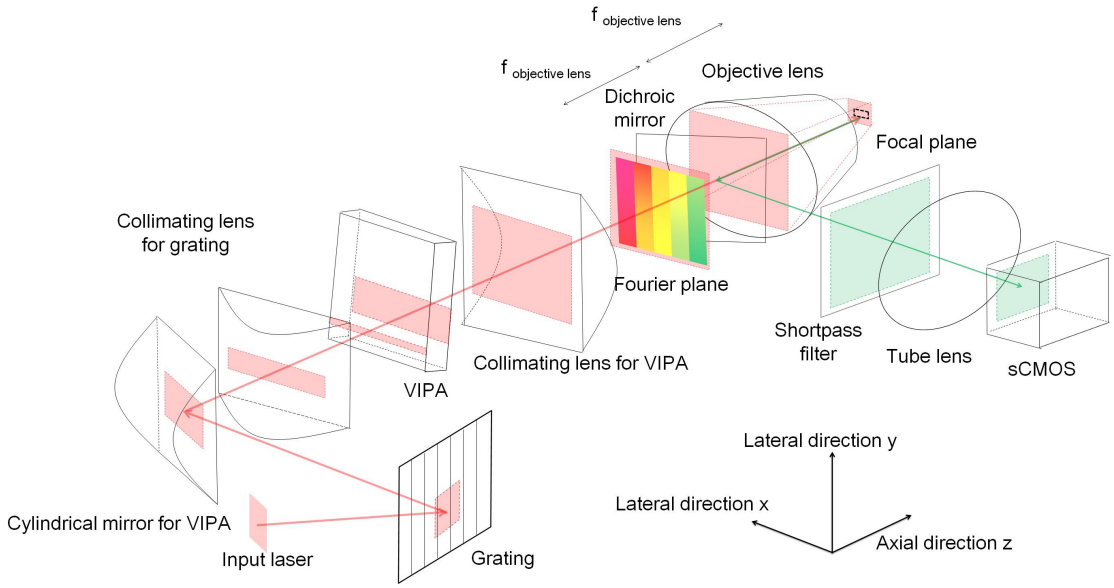

Figure 1. Setup of 2D SSTF microscopy

3. EXPERIMENTS AND RESULTS

3.1 Contrast improvement of 2D SSTF

We first prepared the tissue-like phantom and get the 3D image of it. The tissue-like phantom contains 2μm fluorescent polystyrene bead solution. The mean free path length of excitation light is chosen around 100μm by controlling the density of bead solution. The sample is scanned axially to acquire the 3D image. Since the amount of ballistic light decreases exponentially along the axial direction when the light penetrates inside the sample, we increase the laser power to compensate this attenuation. Figure 2 shows the sectioning image around the surface and the inside of the sample acquired by SSTF and 2D SSTF when we set the similar Raleigh length for the both. The contrast at surface is almost the same. However 2D SSTF performs better than SSTF especially inside the sample.

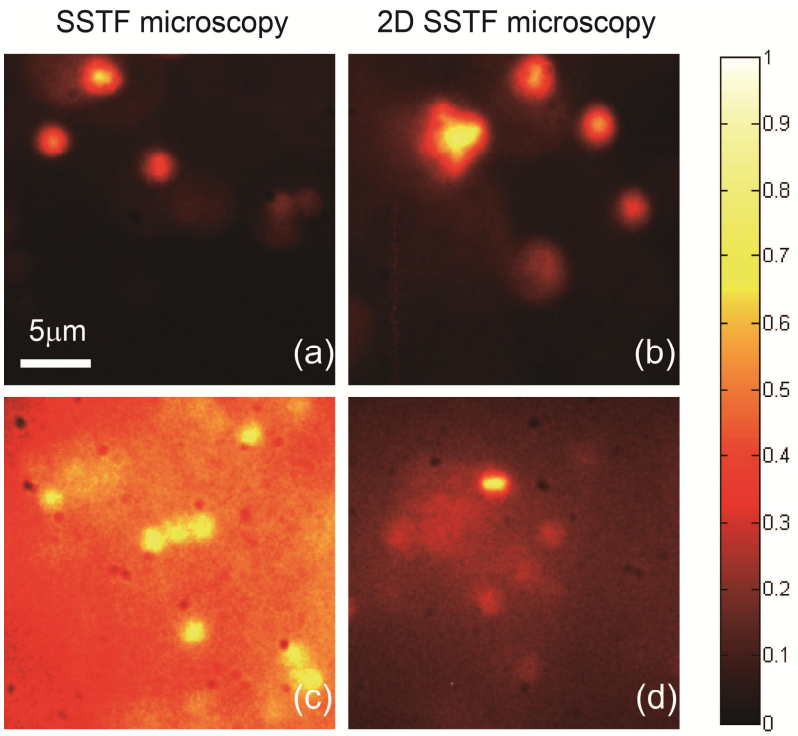

Figure 2. Comparison of the contrast between SSTF microscopy and 2D SSTF microscopy: image at the surface of the sample for (a) SSTF microscopy and (b) 2D SSTF microscopy; image inside the sample for (a) SSTF microscopy and (b) 2D SSTF microscopy.

3.2 3D Brownian motion measurement

In order to achieve high speed volume imaging, a scientific CMOS (sCMOS) camera and a piezo stage are integrated for the imaging system. Every 2D image size is 128 by 216 pixels for a 1 kHz frame rate, which is limited by the acquisition time of our sCMOS camera. We capture a volume image of 17.8 μm x 10.6 μm x 5.4 μm (z) at 50 volumes per second by scanning in z direction. The Brownian motion of a 1 μm fluorescent bead is traced. The root mean square (RMS) displacement of Brownian motion is proportional to $\sqrt{\Delta t}$ where $1/\Delta t$ is the volume rate [6]. Our system can support a RMS displacement around 240 nm according to the calculation. It is less than half of the radius of the bead which make us able to trace the random trajectory of Brownian motion. Figure 3. (a)-(c) show the measured displacements in X, Y and Z direction in a duration of 3 sec. A weighting algorithm is used to determine centroid of bead with sub-pixel resolution. The RMS displacement is measured as 226 nm, which agrees the numerical calculation very well.

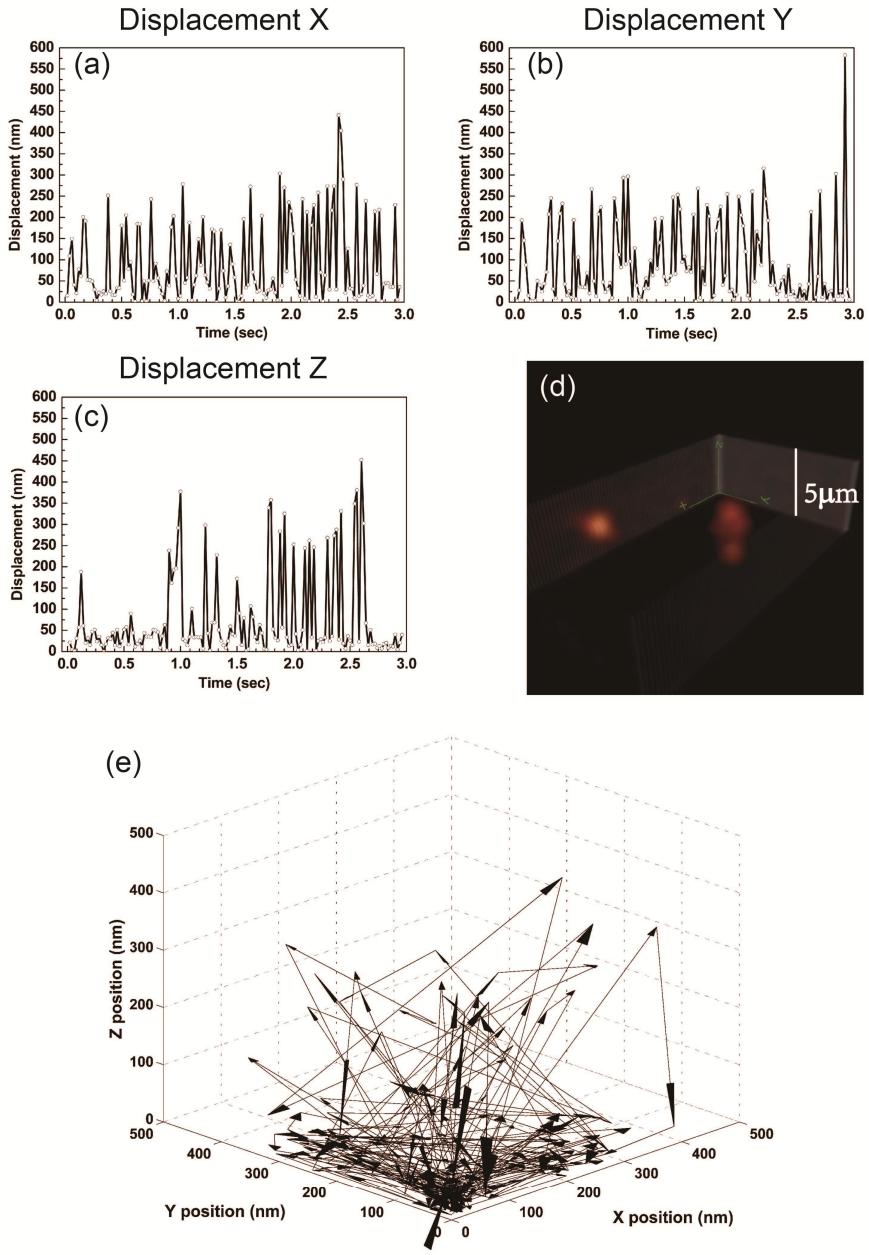

Figure 3. Brownian motion measurement: the displacement of beads in (a) X, (b) Y and (c) Z direction; (d) the volume image at the start of measurement and the target bead is the one in the left part of the image; (e) the trajectory of Brownian motion.

4. SUMMARY AND OUTLOOK

In summary, 2D SSTF technique improved sectioning ability because of less out-of-focus excitation. And this improves the imaging contrast inside the sample. A 50Hz-volume-rate 2D SSTF microscopy was built and 3D Brownian motion detection was demonstrated. As the drawback of using parallel detector, emission scattering will cause the cross-talk noise in the camera. HILO method and structured illumination method can solve this problem [7,8]. In the future we can combine 2D SSTF technique with these methods to achieve deeper imaging with fast volume rates.

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