Axial scanning by pulse shaper for simultaneous spatial and temporal focusing of femtosecond laser pulses

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Abstract

We develop a wide-field simultaneous spatial and temporal focusing (SSTF) setup for two-photon excited fluorescence microscopy and its axial focusing point is scanned by second-order dispersion added by a femtosecond laser pulse shaper placed before the setup. We measure the axial resolution of second harmonic generation (SHG) at the edge of a BBO crystal.

1. Introduction

Simultaneous spatial and temporal focusing (SSTF) is proposed and realized in 2005 [1,2]. Compared with point-scanning or line-scanning multi-photon microscopy, it's possible to obtain full-frame depth resolved imaging completely without scanning in the x-y plane [2]. On the other hand, it shows the great potential to significantly reduce the background excitation in multi-photon microscopy which limits the imaging depth in highly scattering biological specimens [1]. Zhu developed the analytical expressions to describe this method [1], while Oron demonstrated the axial resolution of 4.5 μm for SSTF two-photo microscopy which is comparable to that of line-scanning two-photon microscopy [2]. Later Dust showed that SSTF can linearly scan the temporal focal plane axially by adjusting the group velocity dispersion [3]. This shows a highly promising application, axial scanning multi-photon fluorescence fiber probe without any moving parts at the distal end [3].

In our work, first we built a wide-field SSTF setup and verified its axial resolution by a differential SHG detection scheme [4] where the axial resolution of the second harmonic signal generated at the edge region of BBO crystal is measured. Then, we used a femtosecond laser pulse shaper to add the group delay dispersion (GDD) in order to scan the temporal focal plane axially.

2. Experimental setup

We built an SSTF setup shown in Fig. 1. We used a mode-locked Ti:Sapphire laser (Venteon) with an average power of 130mW and a repetition rate of 150 MHz as a light source. The bandwidth is 450 nm and the central wavelength is 800nm. In the present SSTF setup, not all the spectrum was used. The actual bandwidth we used was 70 nm, (800- 870 nm). The actual laser power after the objective lens was 4-6 mW. The laser beam was first sent to a pulse shaper consisting of a couple of grating and lens, and a liquid crystal spatial light modulator LC-SLM). Then, the laser beam was angularly dispersed with a diffractive grating (*G*=150/mm) and collimated by the mirror with a focal length of 400 mm. Finally, the beam was focused by the objective lens (Olympus UPLSAPO 40X) with focal a length of 4.5 mm and a numerical aperture of 0.95 into a 300-μm thick BBO crystal. The second harmonic generation signal was extracted by a dichroic mirror and detected by the photo-multiplier.

In order to calibrate the distance between grating and focusing mirror to cancel the geometric dispersion, we use a spatial spectral interferometer (SSI) to measure the spectral phase and tune the distance until there is no dispersion [5]. Figure 2 shows the SSI measurement result when there was no dispersion.

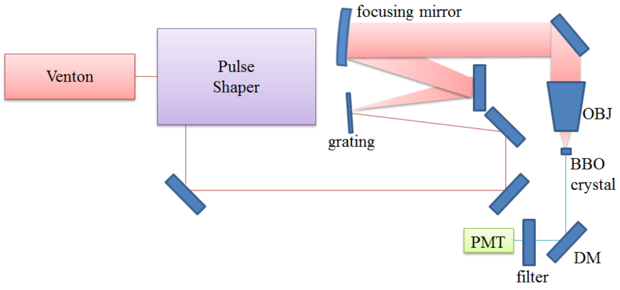
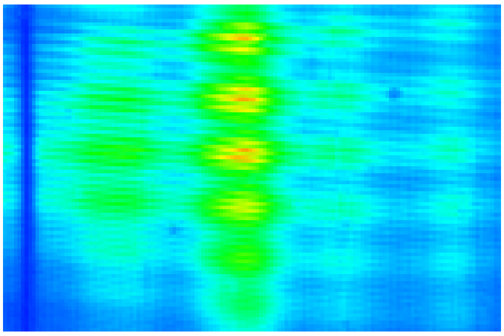
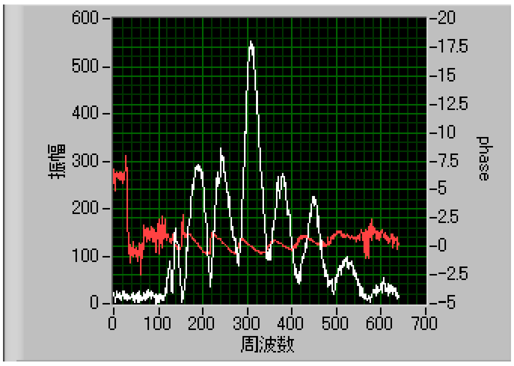


Fig. 1. SSTF experimental setup. Venteon: mode-locked Ti:Sapphire laser. OBJ: objective lens (Olympus UPLSAPO 40X). DM: dichroic mirror. PMT: Photomultiplier tube.

Note that the saw-like phase was the measurement error came from the background interference pattern.



(a)



(b)

Fig. 2 SSI measurement result (a) SSI pattern (b) calculated phase and spectrum from the pattern.

In order to optimize the distance between objective lens and focusing mirror, we used the zero-order diffracted light and measure the central power one meter away from the objective lens by a CCD camera. Once we tune the distance to get the highest central power, the distance is optimized which means the diffraction effect for each mono-chromatic beam is lowest in SSTF setup.

3. Results

First, we compensate for the dispersion generated by objective lens and other possible optics in the SSTF system by pulse shaper. We used the zero-order diffraction light and measure the SHG signal. We tune the group delay dispersion (GDD) by pulse shaper to get the highest SHG signal. Figure 3 shows the relation between GDD and SHG signal.

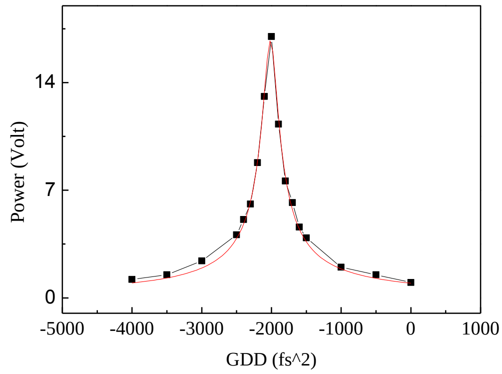
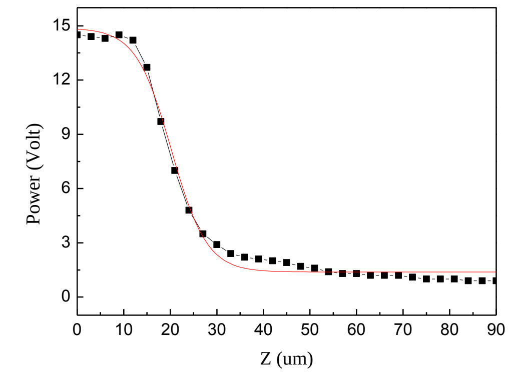


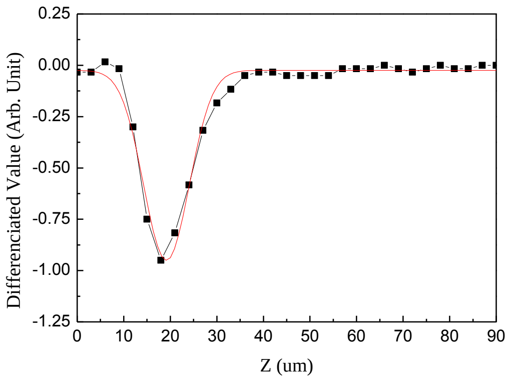
Fig. 3 SHG signal power and GDD relationship

Then, we used the axial edge scheme to measure the SHG signal generated by the SSTF setup and estimate the axial resolution of SSTF setup. Note that the SHG resolution is the same as the two-photon excitation resolution for two-photon microscopy since these are only determined by the amplitude and phase distribution of the laser beam. Figure 4 shows the result. The axial resolution of our system is 15 μm. Note that the dispersion of the BBO crystal itself and the refraction is the error source for this measurement.

Finally, we tune GDD by the pulse shaper to axially scan the focal plane. For every GDD value, we measured the axial position of the middle point of SHG signal using the axial edge scheme. Figure 5 shows the result. It shows good linearity between axial position of focal plane and added GDD value. Note that there are



(a)



(b)

Fig. 4. Axial resolution measurement by axial edges: (a) direct measurement by BBO crystal; and (b) differentiated value from (a).

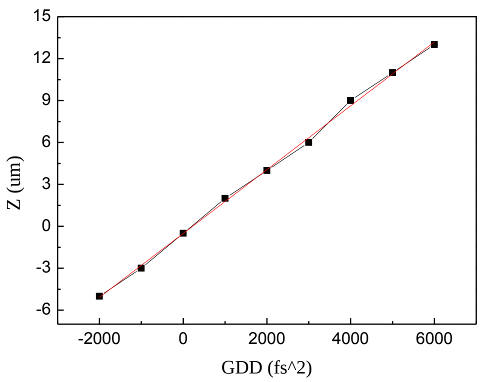


Fig. 5. Change in axial position of the focal plane by GDD added to the excitation laser pulses.

more errors when the GDD is larger due to the limitation of the pulse shaper.

4. Conclusion

We succeeded in building the wide-field SSTF setup. We verified the axial resolution by using axial edge scheme of a BBO crystal. The axial resolution of our setup is 15 μm. Furthermore, we succeeded in tuning GDD by pulse shaper to linearly scan the focal plane.

Reference

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