Characteristics of point-focus Simultaneous Spatial and temporal Focusing (SSTF) as a two-photon excited fluorescence microscopy

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Abstracts: We theoretically and experimentally verified that the sectioning ability for the point-focus simultaneous spatial and temporal focusing (SSTF) microscopy is the same as the point scanning TPE microscopy.

1. Introduction

A SSTF microscopy utilizes the tilted pulse-front to achieve ultrafast scanning up to the repetition rate of the laser source. At the same time, the temporal focusing effect of SSTF offers the system the axial confinement ability similar to a line-scanning TPEF microscopy. [1,2] Since when we use scanning confocal microscopy to generate TPEF image, the axial confinement ability is improved, we have the similar idea to combine the technique of point-scanning TPEF microscopy with SSTF. [3]

In our work, we design the setup to generate a spatial chirped beam among which every single wavelength component has a big enough beam size to fulfill the back aperture of the objective lens. Finally, the result shows no improvement for the sectioning ability of this point-focus SSTF microscopy. In theory, we review the model of SSTF and find out that a point-focus SSTF microscopy will effectively decrease the bandwidth and thus the temporal focusing effect is lower than the point focusing effect. However, if we don't fully utilize the N.A. of the objective lens, we can weaken the degradation of bandwidth. On the other hand, the point focusing effect is lower due to degraded N.A.. So to some extent, the spatial focusing effect is lower than the temporal focusing effect and thus we can find the relative improvement of sectioning ability.

2. Experimental setup

As Figure 1 shows, a mode-locked laser (MIRA) with an average power of 500mW and a repetition rate of 76 MHz works as a light source. The bandwidth is around 40 nm and the central wavelength is 810nm. Modulated by the pulse shaper and attenuated by the neutral density (ND) filter, the laser source has an average power more than 40mW. By the first concave mirror, Laser is focused on the surface of a grating whose groove number is 600 per millimeter. Then the light is collimated by the second concave mirror and the spatial chirped light is collimated. After objective lens, we set the moving stage to hold the crystal silica (birefringence filter) as a sample to generate surface second harmonic (SHG) light whose. For the moving stage where we hold the sample, we can move it in axial direction by a close-loop pico-motor (New Port) as well as a hand-moving mechanical x-stage (Sigma Koki). SHG light is collected by PMT after one DM, one filter and collecting lens. In order to prevent the noise from background light, we use Lock-in detection method to measure the SHG intensity along axial direction. Thus we put a chopper (3501 optical chopper, New Focus) with 1 KHz chopping frequency just before the setup in Figure 1 but after the pulse shaper.

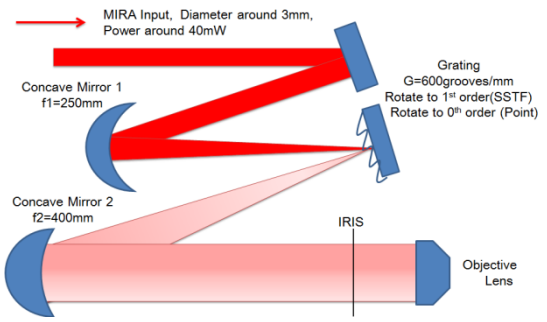
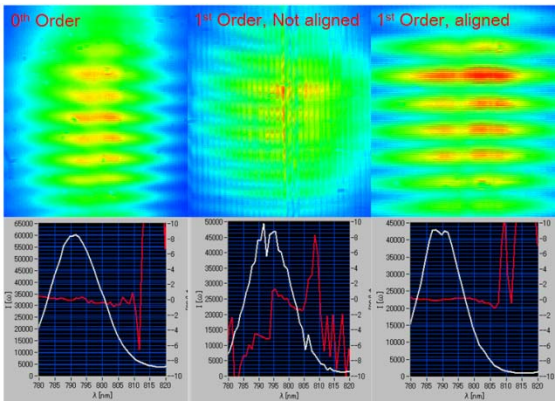


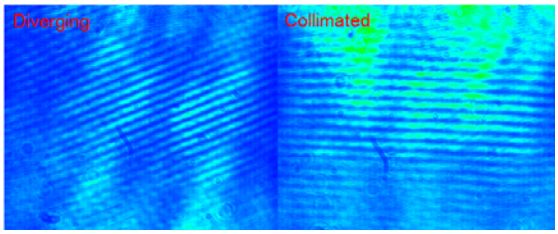
Figure 1 Setup of point-focus SSTF microscopy.

We first use spatial spectral interferometry (SSI) to fine align the distance between grating and

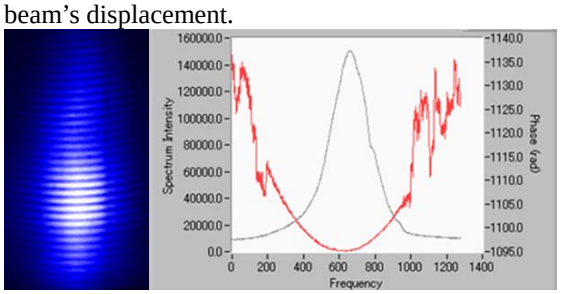
second concave mirror in order to confirm the distance is exactly a focal length. [4] The experimental interferometry patterns was captured under a not-well aligned condition where the horizontal (spectrum coordinate) incident angle is not fine-aligned to the same angle, thus only when we move the CCD camera away the focal length, we can see the relatively clear pattern. Also due to this, the jump in spectrum phase occurred. Figure 2 (c) shows another SSI experimental pattern when we aligned pulse shaper. At that time, we first fine-align the distance between camera and collimating lens for SSI by checking the zero-order diffraction image of only signal light to be the thinnest line. Then we use first-order diffraction light and tune the horizontal incident angle of reference light to search the fine interference pattern. Please note that we have to make the holder of grating to keep the rotation axis locating at the surface of grating. Next, we use a shearing interferometer and CW mode of the mode-locked laser to fine-align the distance between first concave mirror and grating in order to keep the monochromatic beam after second concave mirror as a parallel beam. [5] In figure 2 (b), the signal beam and reference beam are separated in horizontal direction and the shearing angle is in vertical direction.



(a) SSI interference pattern; left: we use zero-order diffraction light in (a); center: we use first-order diffraction light while the distance is not aligned to be a focal length; right: we use first-order diffraction light when the distance is fine-aligned; For the three figures in bottom is the processed spectrum amplitude (white line, left bar) and phase (red line, right bar), frequency is under arbitrary unit



(b) Interference pattern of shearing interferometer; left: when the beam is not collimated, the direction of fringes is titled; right: when the beam is collimated to a parallel beam, the direction of fringes is parallel to the direction of reference beam's displacement.



(c) SSI interference pattern; left: the pattern; right: the processed spectrum amplitude (gray line, left bar) and phase (red line, right bar), frequency is under arbitrary unit

Figure 2 Results of alignment

3. Experiment Results

3.1 Lock-in detection for axial FWHM of SHG intensity

We connect the signal from PMT to the digital lock-in amplifier (SR830, Stanford Research Systems). Meanwhile, we parallel-connect the PMT to an oscilloscope (Tektronix) with an input impedance of 1MΩ in order to balance the input impendence of the measuring system as well as to check the original PMT signal. Since we have to measure the same condition for objective lens with different N.A., once we change the objective lens, we have to re-align it or check the alignment. Before installing the objective lens, we can first align the incident light parallel to the optical table and then we mark the center of the light very close to the focal point by a white paper. Note that in order to accurately know the position of the center, we can use a closed iris. Then we install the objective lens and tune the holder in vertical and horizontal direction in order to make the focal point locate at the marking position. Since the light is hard to see directly by eyes, we can utilize the IR viewer to check the scattering laser pattern on the

paper. Finally, we hold the sample and put the polarization direction where we know from previous experience to maximize the SHG power. Since the crystal silica sample has birefringence, it shows sinusoid change for the SHG intensity by changing the polarization angle. To check the signal on oscilloscope, we can search the front surface where the signal suddenly increase and decrease with moving the stage by hands. Please note that the back surface of the sample also generate SHG light.

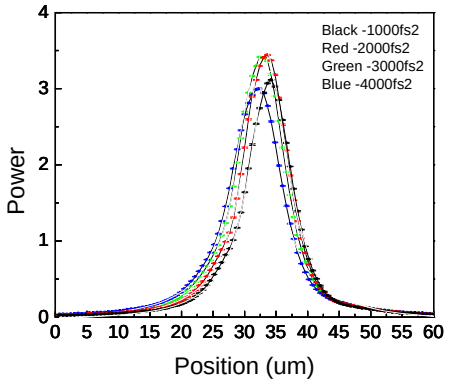
For the measurement, we use the synchronized output signal from chopper as the reference signal to the digital lock-in amplifier. The time constant is set as 100msec or 300msec with 24dB/oct rolloff in order to trade-off between the measuring time and measuring accuracy. We tried different objective lens with different beam size which is controlled by an iris. Every time, we measure the surface SHG intensity by scanning the crystal along the axial direction for both point-SSTF and point scanning microcopy. In order to achieve that, we switch the grating between first-order diffraction and zero-order diffraction and then re-align the angle of grating by checking the beam position on the iris. Please note that the surface of grating is on the rotating axis. The results of axial resolution of SHG intensity are listed in Table 1. There are no improvements for point-SSTF microscopy when the size of the monochromatic beam fully covers the back-aperture of objective lens. Only when the size is smaller than the back-aperture of objective lens, we can achieve some improvements.

Table 1 Axial resolution of SHG intensity

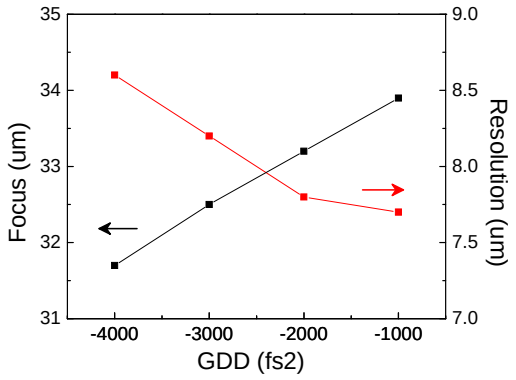
Type	N.A.=0.95	N.A.=0.45	N.A.=0.40
Point(Full)	6.7um	4.4um	7.8um
Point(Part)	8.6um	5.5um	17um
P-SSTF(Full)	6um(90%*)	4.4um(100%)	7.8um(100%)
P-SSTF(Part)	7.4um(86%)	4.6um(84%)	9.2um(54%)

The percentage in the brackets means the percentage of the axial resolution of point scanning type. Full means the monochromatic beam size is fully utilized while part means the monochromatic beam size is partly used. *For the case N.A.=0.95, the full size of monochromatic beam is less than the back-aperture of objective lens while for other cases, the full size of monochromatic beam is bigger than the back-aperture of objective lens

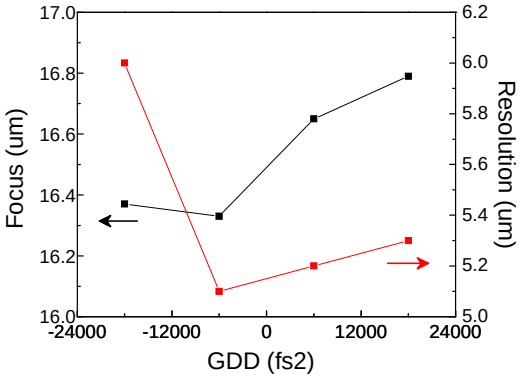
We also measure the shift of focal plane and degradation of axial resolution due to additional dispersion. As Figure 3 shows, there are both changes in axial resolution and focus position. For the shift of focal plane, there is shift and its direction always follows theory while the shifting value is lower than the theoretical value for most of the case when the monochromatic beam is either fully or partly used. [6] The main reason is that there is a competition between temporal focusing and point focusing, once the focus of them are different, the final focus is mainly determined by the point focusing due to the degraded focusing power of temporal focusing which is explained in Part 3.2. Moreover, due to the same reason, the axial resolution will degrade when the focus of temporal focusing and point focusing is separated. We think even the detail of the beam profile in axial direction for point focusing and temporal focusing will influence on these issues. Part 3.3 will discuss about this.



(a) The SHG intensity distribution along axial direction for different additional dispersion when the objective lens has an N.A.=0.40 and monochromatic beam is fully used.



(b) The fitting result of (a)



(c) The fitting result for an objective lens with N.A.=0.45 when its monochromatic beam is partly used

Figure 3 Focus shift and resolution with dispersion

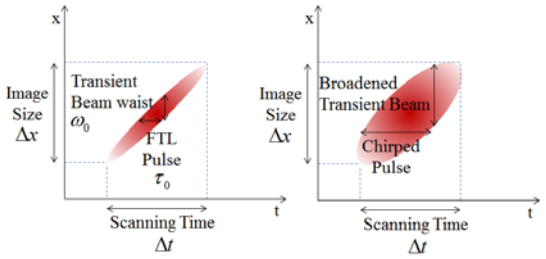
3.2 Theoretical analysis

For the basic theory of SSTF, we know the beam at the focal plane is [6]

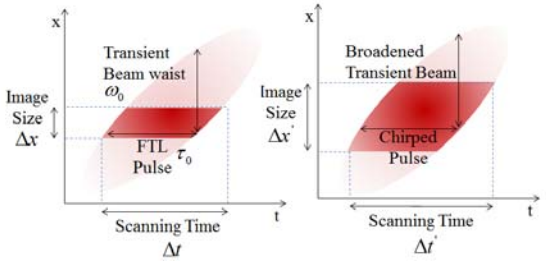
$$E(t,x) = A(t - Delay, x) \quad Delay = \frac{2\pi}{\lambda_c}\gamma x \qquad \ldots\ldots\ldots(1)$$

$$A(t,x) = A(t) \times A(x) \qquad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Here γ is the ratio between them which is determined by the specification of grating and ratio between the focal length of collimating lens and objective lens and λ_c is the central wavelength. Please note that here we only consider the lateral direction where the angular dispersion occurs. For another perpendicular lateral direction, it's independent and only has spatial focusing. Then only difference for point-SSTF is that the function A(x) becomes a tightly focused beam profile rather than a big plane wave and thus it will quickly broaden with axial position. On the other hand, if we consider the pupil size of objective lens, the frequency component will be partly blocked due to the big monochromatic beam size at the back-aperture of objective lens and the displacement of different frequency component. When the size of monochromatic beam is larger, the loss is bigger. We can regard this loss as a decrease of bandwidth for angular frequency. In time domain, this means the increase of temporal duration which causes a broadening of transient beam waist. So in sum, the final axial confinement ability will be determined by the faster broadening process. In another words, we need to compare the size of transient beam and the size of focused monochromatic beam A(x). For the case when monochromatic beam is bigger than the back-aperture of objective lens, the size of focused monochromatic beam is minimized while the transient beam size is maximized due to the loss of bandwidth. For the case when monochromatic beam is smaller than the back-aperture of objective lens, the size of the focused monochromatic beam is broadened while the transient beam is smaller than the first case. If we continue to decrease the size of the monochromatic beam, we will further increase the size of focused monochromatic beam and further decrease the size of transient beam similar to a regular SSTF microscopy. Figure 4 shows this difference. Image size is the beam size of focused monochromatic beam. In order to give you the image of how transient beam broaden, it shows the virtual full SSTF beam whose image size is much bigger than the transient beam size in the point-SSTF case. Finally, the focusing power of monochromatic beam is in two dimensions while the temporal focusing power is only in one dimension. This will further enhance the dominant position of monochromatic beam focusing.



(a) Regular SSTF, left at focus, right outside focus



(b) Point-SSTF, left at focus, right outside focus; the weak red part is the virtual full SSTF beam

Figure 4 Spatial-temporal amplitude distributions

3.3 Influence of axial beam profile

In the experiment, we sometimes find the axial SHG intensity distribution is not a symmetric one

which means the axial beam profile is not symmetric. This will influence on the axial resolution and also the shifting ability of focus via additional dispersion. There are several possible reasons for the asymmetric shape. The first one is that the focus of the monochromatic beam is not at the same axial position as the temporal focus. The second one is that even when they are at the same position, the chromatic dispersion of the lenses has the potential to break the symmetric shape. The third one is that the use of concave mirror will lead a displacement of the monochromatic beam's focus in horizontal and vertical direction which is called astigmatism. The fourth one is any other image aberration due to the objective lens we are using. For example, the common case is mismatch of anti-reflection coating and spherical aberration. [7] The final one is the miss-alignment of objective lens. All of these five factors can change the axial beam profile to an asymmetric case or broadened case.

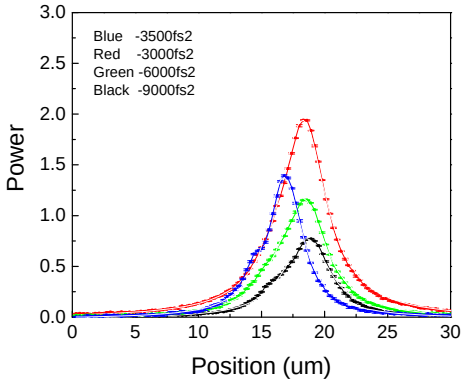


Figure 5 Axial SHG intensity distributions

Here I am going to use an interning example to illustrate the influence on point-SSTF microscopy. Although I am not sure which certain factor causes this asymmetric shape, in Figure 5, it shows you the change of the shape once I change the additional dispersion. And at the same time, the axial resolution as well as the focus shifting rate is changing. The change of the axial resolution mainly comes from two parts. One is the focus position of temporal focusing and point focusing as pointed in part 3.2. Another part is the axial beam profile. If the temporal focus position is close to the monochromatic beam focal region and is within the middle of main peak and side peak of the profile, it will give us a symmetric shape as well as a slightly broadened shape which is the case of red line in Figure 5.

4. Conclusion

We tried point-SSTF microscopy by using tightly focused monochromatic beam rather than big plane wave of regular SSTF. There is no improvement when we fully utilize the monochromatic beam size. Additionally, the change of focus position due to additional dispersion is much smaller than regular SSTF while the axial resolution will be changed by the additional dispersion value.

Reference

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