Characterization of a broadband pulse for phase controlled multiphoton microscopy by single beam SPIDER

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We present what we believe to be a new version of spectral phase interferometry for direct electric field reconstruction (SPIDER) using only a single-phase and polarization controlled laser beam. Two narrow pulses and one broadband pulse are selected out of an ultrafast laser pulse by a polarization and phase control technique to generate second harmonic generation (SHG) signals, which are equivalent to a spectral shear interferogram in the conventional SPIDER method. The spectral phase of the broadband laser pulse is extracted analytically with double quadrature spectral interferometry (DQSI). An arbitrary spectral phase can be retrieved with great precision and compensated *in situ* at the sample position of a microscope. This new method requires no separate reference beam and is suitable for nonlinear optical microscopy with a phase controlled laser pulse.

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Recently, there has been significant interest in multiphoton microscopy utilizing phase controlled ultrafast laser pulses [1–5]. Numerous novel concepts have emerged in relation to the quantum control of light-matter interaction; these open up new possibilities such as chemical selectivity [1–3], high image contrast [4], and vertical sectioning [5]. To apply these novel nonlinear optical schemes to high resolution optical microscopy, it is very important to compensate for the spectral phase distortion of the ultrafast pulse at the sample position of a microscope [6,7]. In multiphoton microscopy, a high numerical aperture microscope objective lens introduces significant high order spectral phase distortions due to the elaborate multiple optical components inside, and the dispersion is mostly unknown. It is desirable to retrieve the exact shape of the spectral phase at the sample position and compensate for the spectral phase distortion *in situ* if one wishes to exploit the full potential of the phase controlled laser pulse.

There are several techniques to compress an ultrafast pulse at the microscopic sample position. The most popular one is the adaptive pulse compression method to maximize second harmonic generation (SHG) or other nonresonant nonlinear signals [8]. Although this approach can compress ultrafast pulses, it is an indirect pulse characterization method, and there is no guarantee that the obtained phase information is exact over the entire laser bandwidth. Several direct spectral phase characterization techniques have also been introduced recently with a multiple beam combination or scanning sinusoidal phase mask [6,7,9].

Here we present what we believe to be a novel direct pulse characterization method requiring only a single laser beam. It is a new version of spectral phase interferometry for direct electric field reconstruction (SPIDER) that can retrieve and compensate for the spectral phase at the sample position with only a single-phase and polarization controlled laser beam. This single beam SPIDER method shares the spectral shear interferometry concept with the conventional SPIDER. However, we apply the double quadrature spectral interferometry (DQSI) method instead of Fourier transform spectral interferometry (FTSI) to make the single beam technique possible [10]. It has a simple experimental setup and is ideal for characterizing the phase structure of a broadband laser pulse in multiphoton microscopy.

SPIDER is a well established phase characterization technique that can retrieve an exact form of the spectral phase directly [11]. Briefly, two time-separated replicas of a target pulse are combined with a strongly stretched reference pulse in a nonlinear crystal to generate two replicas of SHG signals that are slightly frequency-shifted to each other. The two SHG signals generate a spectral shear interferogram, from which the spectral phase of the target pulse is retrieved by FTSI [11].

One can make such a spectral shear interferogram with a single-polarization and phase controlled laser pulse. A cavity dumped Ti:sapphire laser pulse (Cascade, KM Labs) is polarization and phase controlled by an all reflective 4*f* pulse shaper with a dual mask 640 pixel liquid crystal spatial light modulator (SLM-640, CRI) (Fig. 1(a)) [12]. We operate the pulse shaper in the polarization and phase control mode by removing the exit polarizer for the SLM [12]. Two linearly polarized (*y*-axis) narrow bandwidth probe pulses (Pr_1 and Pr_2) are selected by applying the polarization and phase masks as shown in Fig. 1(b). The bandwidths of probes (Pr_1 and Pr_2) are 1.6 nm (4 SLM pixels) and are separated in frequency by 4 nm (10 SLM pixels, center to center). In this way we have one short and two long pulses in the *x*- and *y*-polarization directions, respectively. Note that a similar approach has been introduced with separate reference beams [9]. Here we implement the same

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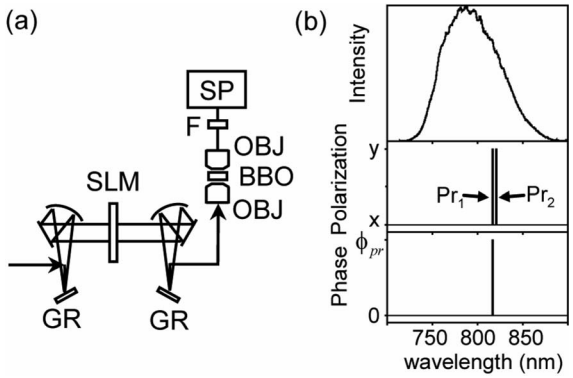

Fig. 1. (a) Experimental setup: GR, grating; SLM, dual-bank liquid crystal spatial light modulator; OBJ, microscope objective lens; F, BG28 filter; SP, spectrometer. (b) Spectrum of the laser (top). Polarization (middle) and phase (bottom) masks used in the experiment.

concept with only a single shaped laser pulse. This pulse is then focused onto a 100 μm thick BBO crystal with a type II phase matching condition by a 0.65 NA microscope objective (Olympus). The SHG signal is collected by another objective (0.4 NA Olympus), filtered by a BG 28 filter and measured with a minispectrometer (USB4000, Ocean Optics). The pulse energy is 0.5 nJ before the objective lens and the spot size at the focus is 1.4 μm (FWHM). Since we generate SHG signals with two narrowband y-polarized pulses and one x-polarized broadband pulse in a type II phase matching condition, there are two separate SHG signals whose frequencies are shifted by the frequency interval between the two probes [Pr_1 and Pr_2 in Fig. 1(b)]. The amplitude and phase of the two SHG electric fields are expressed as

$$E^{(1)}_{SHG}(\omega+\omega_p+\delta\omega) \propto E^0(\omega_p+\delta\omega)E^0(\omega) \times \exp(i\phi(\omega)+i\phi_{pr}),$$

$$E^{(2)}_{SHG}(\omega+\omega_p) \propto E^0(\omega_p)E^0(\omega)\exp(i\phi(\omega)),$$

where $E^{(1)}_{SHG}(\omega)$ and $E^{(2)}_{SHG}(\omega)$ are the SHG electric fields at the frequency ω by mixing the x-polarized pulse with Pr_1 and Pr_2, respectively. $E^0(\omega)$ is the field amplitude of the original laser pulse. $\omega_p+\delta\omega$ and ω_p are the frequencies of Pr_1 and Pr_2, respectively. $\phi(\omega)$ is the spectral phase of the x-polarized broadband pulse, which one wishes to retrieve. We set the phases of Pr_1 and Pr_2 to be ϕ_{pr} and zero, respectively (Fig. 1(b)). Note that we can control the relative phase difference between the two SHG signals by ϕ_{pr}.The measured SHG spectrum is a coherent sum of the two SHG fields and is expressed by

$$\begin{aligned} S(\omega+\omega_p) &= |E^{(1)}_{SHG}(\omega+\omega_p)+E^{(2)}_{SHG}(\omega+\omega_p)|^2 \\ &\propto |E^0(\omega_p+\delta\omega)E^0(\omega-\delta\omega)|^2 \\ &\quad + |E^0(\omega_p)E^0(\omega)|^2 \\ &\quad + f(\omega)(\exp[i(\theta(\omega)-\phi_{pr})] \\ &\quad + \exp[-i(\theta(\omega)-\phi_{pr})]), \end{aligned} \tag{1}$$

where $f(\omega)\equiv E^0(\omega_p+\delta\omega)E^0(\omega-\delta\omega)E^0(\omega_p)E^0(\omega)$ and $\theta(\omega)\equiv\phi(\omega)-\phi(\omega-\delta\omega)$. $f(\omega)$ and $\theta(\omega)$ correspond to the amplitude and phase of the cross term, respectively. Note that only the interferometric cross term depends on the phase of Pr_1 (ϕ_{pr}). Then it is straightforward to show that $\theta(\omega)$ and $f(\omega)$ can be obtained by measuring four SHG signal traces with $\phi_{pr}=0$, $\pi/2$, π and $3\pi/2$ by the following relations:

$$\theta(\omega)=\tan^{-1}\left[\frac{S(\omega+\omega_p,\phi_{pr}=\pi/2)-S(\omega+\omega_p,\phi_{pr}=3\pi/2)}{S(\omega+\omega_p,\phi_{pr}=0)-S(\omega+\omega_p,\phi_{pr}=\pi)}\right], \tag{2}$$

$$f(\omega)=\frac{1}{4}\sqrt{(S(\phi_{pr}=0)-S(\phi_{pr}=\pi))^2+(S(\phi_{pr}=\pi/2)-S(\phi_{pr}=3\pi/2))^2}. \tag{3}$$

Note that we use DQSI instead of FTSI to extract $\theta(\omega)$ [10]. This is the main difference in the current method from the conventional SPIDER [11] and makes the single beam technique possible. From $\theta(\omega)$ one can get the original spectral phase by [11]

$$\phi(\omega_0+n\,\delta\omega)=\phi(\omega_0)+\sum_{k=1}^{n}\theta(\omega_0+k\,\delta\omega), \quad \text{where } n \text{ is a positive integer}, \tag{4}$$

ω_0 is the lowest frequency of interest, and a linear spectral phase (group delay) is removed in the retrieved $\phi(\omega)$. Note that the spectral resolution of this technique is determined by $\delta\omega$, the frequency difference between Pr_1 and Pr_2.

Figure 2(a) shows four measured SHG spectra with $\phi_{pr}=0$, $\pi/2$, π, and $3\pi/2$. One can see that the spectral interference pattern changes with ϕ_{pr} dramatically. Note that the original laser pulse is strongly chirped by the microscope objective. Figure 2(b) shows the amplitude [$f(\omega)$] and phase [$\theta(\omega)$] of the interferometric cross term retrieved with Eqs. (2) and (3). Also note that the x-polarized pulse spectrum has two spectral holes where we select the two y-polarized probes. This leads to the three notches in the retrieved amplitude spectrum of the cross term [$f(\omega)$] in Fig. 2(b).

There is one technical problem in this new SPIDER technique. Even though we use a type II phase matching condition, there may be some type I phase matching SHG backgrounds generated by the

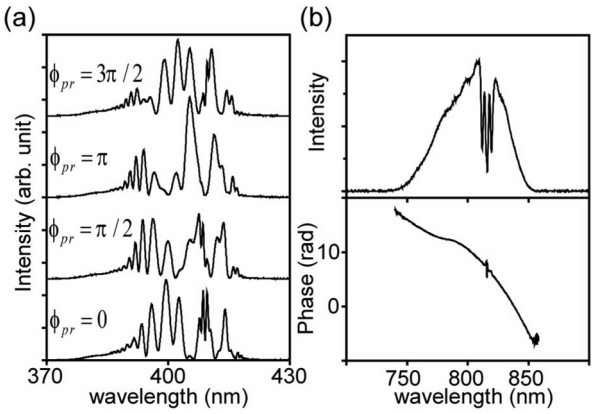

Fig. 2. (a) SHG spectra with different phases at Pr_1 (ϕ_{pr} $=0$, $\pi/2$, π and $3\pi/2$). Each trace is displaced vertically for clarity. (b) Amplitude [$f(\omega)$] and phase [$\theta(\omega)$] spectra of the cross term in Eq. (1) retrieved with Eqs. (2) and (3).

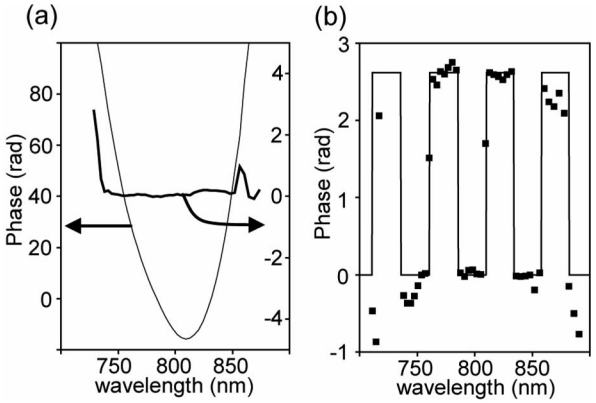

Fig. 3. (a) Spectral phase of the laser pulse at the microscope sample position before (thin curve) and after (thick curve) compressing the laser pulse by applying the compensating phase mask. Note the difference in the vertical scales. (b) Phase mask applied in the SLM (solid curve) and the retrieved spectral phase of the laser pulse by the single beam SPIDER (dot).

x-polarized pulse. This is due mainly to a small birefringence of the microscope objective. The unwanted SHG background interferes with the SPIDER signals to cause significant errors, especially with a shorter pulse. We use the following subtraction method to remove this unwanted background interference.

With the existence of the background, the total SHG signal becomes

$$S = |E^{(1)}_{SHG} + E^{(2)}_{SHG} + E_{bgr}|^2 = |E^{(1)}_{SHG}|^2 + |E^{(2)}_{SHG}|^2 + |E_{bgr}|^2 + 2\,\mathrm{Re}[E^{(1)}_{SHG}E^{(2)}_{SHG}{}^*] + 2\,\mathrm{Re}[E^{(1)}_{SHG}E_{bgr}{}^*] + 2\,\mathrm{Re}[E^{(2)}_{SHG}E_{bgr}{}^*],$$

where E_{bgr} is the electric field of the SHG background. Note that $\mathrm{Re}[E^{(1)}_{SHG}E^{(2)}_{SHG}{}^*]$ has the spectral phase information. $\mathrm{Re}[E^{(1)}_{SHG}E_{bgr}{}^*]$ and $\mathrm{Re}[E^{(2)}_{SHG}E_{bgr}{}^*]$ are the unwanted interference terms from the SHG background. If we select only one probe at Pr_1 or Pr_2, the SHG signals ($S^{(1)}_{bgr}$ and $S^{(2)}_{bgr}$) become

$$S^{(1)}_{bgr} = |E^{(1)}_{SHG}|^2 + |E_{bgr}|^2 + 2\,\mathrm{Re}[E^{(1)}_{SHG}E_{bgr}{}^*]$$

and

$$S^{(2)}_{bgr} = |E^{(2)}_{SHG}|^2 + |E_{bgr}|^2 + 2\,\mathrm{Re}[E^{(2)}_{SHG}E_{bgr}{}^*].$$

Subtracting the sum of $S^{(1)}_{bgr}$ and $S^{(2)}_{bgr}$ from S with the same probe phase (ϕ_{pr}) eliminates the background interferences and the spectral shear interferometric term [$\mathrm{Re}(E^{(1)}_{SHG}E^{(2)}_{SHG}{}^*)$] can be obtained. With the subtraction method, we can eliminate the background SHG interference completely.

Figure 3(a) shows the retrieved spectral phase curves [$\phi(\omega)$] at the microscope sample position before (thin curve) and after (thick curve) compressing the laser pulse. The spectral phase becomes virtually flat over the entire laser bandwidth after compression. Note the difference in the vertical axis scales. Figure 3(b) shows a phase mask of a square wave shape, which we apply in the pulse shaper (solid curve). The spectral phase obtained with the single beam SPIDER method is also shown (dot). We also verified the consistency and accuracy of the current technique by iterating the pulse compression with the single beam SPIDER (data not shown). Typically, the first compression cycle yields a flat spectral phase within 1 rad phase error over the entire spectral range and the accuracy improves at the second or third cycles to reach around 0.3 rad. No further change is observed after the third scan.

In summary we demonstrate a novel spectral interferometric pulse phase characterization method that can be applied to general nonlinear optical microscopy using a pulse shaper. It has a simple experimental setup, is relatively fast (~10 s), and can characterize an arbitrary spectral phase at the sample position of the microscope precisely.

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