Multiphoton Intrapulse Interference 8. Coherent control through scattering tissue

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Abstract: We demonstrate experimentally that selective two-photon probe excitation using phase shaped pulses can be achieved even when the laser propagates through scattering tissue. The pre-optimized phase tailored femtosecond pulses were able to identify acidic and basic solutions of a pH sensitive chromophore hidden behind a slab of scattering tissue. This observation has important implications for future applications of coherent control for biomedical imaging and photodynamic therapy.

OCIS codes: (320.0320) Ultrafast Optics (320.5540) Pulse Shaping (190.7110) Ultrafast Nonlinear Optics

References and links

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1. Introduction

In the last decade a number of reports have demonstrated controlled excitation of atoms and even the controlled photochemistry of isolated molecules [1-3]. More recently these reports have included large organic molecules in solution.[4-7] Phase shaped femtosecond pulses[8] have the potential for influencing the quantum-mechanical laser-molecule interactions, thereby controlling population transfer between different electronic states of large organic molecules.[9-15] Our group has been exploring selective multiphoton excitation of large molecules in solutions[16-18]. The benefits of coherent laser control of large organic molecules are beginning to permeate to the biological field through selective two-photon chemical microenvironment probing[18] and microscopy.[19,20] Here we question if coherent control methods, in particular phase shaping, can be used in situations where the laser transmits through scattering biological tissue.

Coherent control depends on the interference among multiple excitation pathways; this interference is controlled by modulating the phase of the excitation field. Control schemes therefore depend on the precise phase modulation of the laser pulses, which, through constructive and/or destructive interference, optimize the desired outcome and minimize other pathways. The dependence of coherent control on the accurate phase structure of the pulse, however, suggests that these approaches would not be applicable to situations involving transmission through scattering media.

Here we present a study that evaluates the possibility of using coherent control as the laser transmits through scattering tissue. In particular, we evaluate the ability to achieve selective two-photon excitation of a pH-sensitive probe molecule using phase-shaped femtosecond pulses in the presence and absence of scattering tissue. The motivation for the experiment is that coherent control schemes could enhance methods such as two-photon imaging, which has provided images with higher resolution, lower background scattering, and better sample penetration.[21, 22] Selective two-photon excitation could be used to help localize two-photon induced photodynamic therapy (PDT)[23], and restrict its therapeutic effects to diseased tissue, while leaving surrounding tissue undamaged.

2. Experimental section

The setup for the experiment is shown schematically in Fig. 1. The experiment was carried out using a titanium-sapphire oscillator (Kapteyn-Murnane Labs) laser system. The laser spectrum was centered at about 830 nm, with a bandwidth of 90 nm (full width at half maximum), corresponding to 11 fs pulse duration. The average energy per pulse entering the objective was 1 nJ. The laser pulses were calibrated at the sample, after the objective, using the multiphoton intrapulse interference phase scan (MIIPS) method.[18, 24] The spectral phase functions used for this experiment are based on binary phase shaping (BPS), a method that assigns phase values of zero or π to frequency components of the pulse, and is ideally suited for controlling multiphoton transitions.[25] Optimization of the phase structure was achieved by entering the excitation spectra of probe molecule in acidic and basic environments, the characteristics of the laser pulse, and the multiphoton intrapulse interference formalism (MII).[16-18] A learning algorithm was then used to optimize excitation—maximizing the excitation of one form of the probe molecule while minimizing excitation of the other form of the probe (8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) fluorophore in acidic or basic solution, respectively). The two optimized binary phase functions were introduced into the

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pulse shaper. The sample was scanned in the focal plane of the laser, focused by a 5X long-working distance objective. The capillary tubes (1 mm i.d.) were filled with 10^{-3} M HPTS aqueous pH6 and pH10 buffered solutions. A fifth tube was filled with water to serve as a control to demonstrate the absence of signal due to scattered light. The tubes were placed side by side and oriented vertically in a 2 mm quartz cell filled with water. The fluorescence was focused onto a spectrometer with detection wavelength set at 515 nm (the emission wavelength of HPTS) where it was detected and averaged point by point.

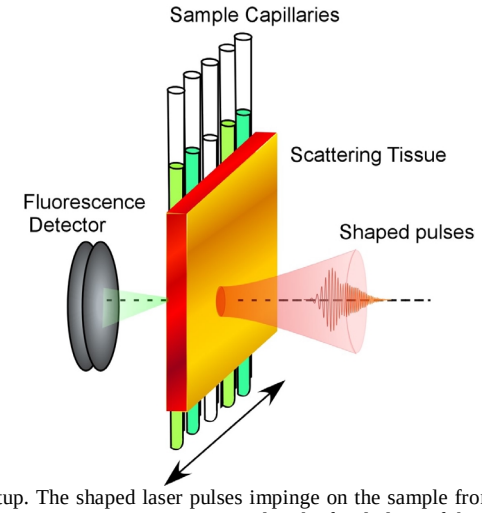

Fig. 1. Experimental setup. The shaped laser pulses impinge on the sample from right to left. The sample with or without scattering tissue is scanned in the focal plane of the laser while the two-photon induced fluorescence is detected at each point.

A 1.5-2.0 mm slice of biological tissue (raw breast of chicken) was placed between the front face of the cell and a microscope slide. The tissue was slightly compressed to a uniform thickness of about 1 mm. A drop of Nujol was used to reduce aberrations caused by index of refraction mismatch. Approximately, 1 mm of tissue is equivalent to 10 scattering lengths.

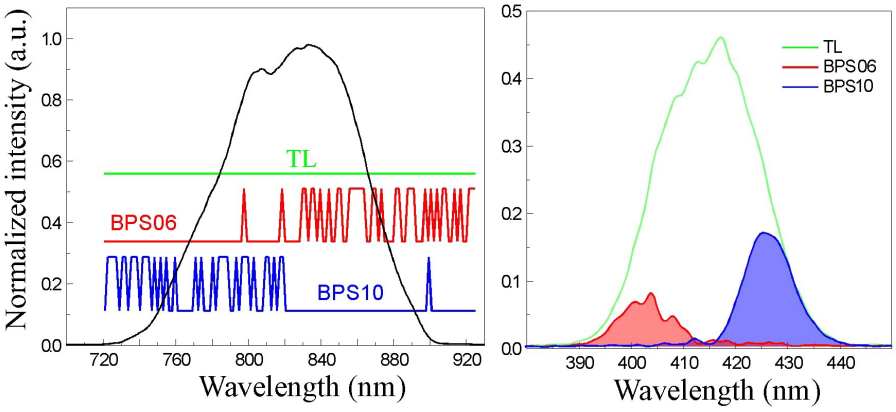

Fig. 2. Optimized phase functions and the resulting SHG after they are tested with a thin SHG crystal. The panel on the left shows the fundamental spectrum of the pulse and the three different pulses evaluated, transform limited (TL), optimized for acidic excitation (BPS06) and optimized for basic excitation (BPS10). The panel on the right shows the SHG spectrum obtained when each of the laser pulses goes through a thin SHG crystal.

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The spectral phase for transform-limited (TL) pulses (green), and the binary phase functions that were used to enhance excitation of HPTS in an acidic (red) or a basic (blue) environment are shown in Fig. 2. The second harmonic generation (SHG) spectrum obtained from a thin nonlinear optical crystal, shown in Fig. 2, provides a diagnostic test of the effects of phase modulation. Unlike the TL pulses, the shaped pulses preferentially excite one of the two different pH solutions. The spectrum of the laser pulse does not change upon phase modulation, only its potential to cause two-photon excitation or generate second harmonic radiation.[16, 17] The change in the SHG spectrum is caused by destructive interference induced by the phase mask.[25]

3. Results

When TL pulses were used, large two-photon induced fluorescence signals at 515 nm, the peak emission wavelength of HPTS in both pH solutions, were observed. The contrast that could be achieved was tested by recording the signal observed when the laser was focused on each of the two HPTS solutions. We carried out this test using two capillary tubes and no scattering tissue. As can be seen in Fig. 3, BPS06 preferentially excites the pH6 solution, and BPS10 preferentially excites the pH10 solution.

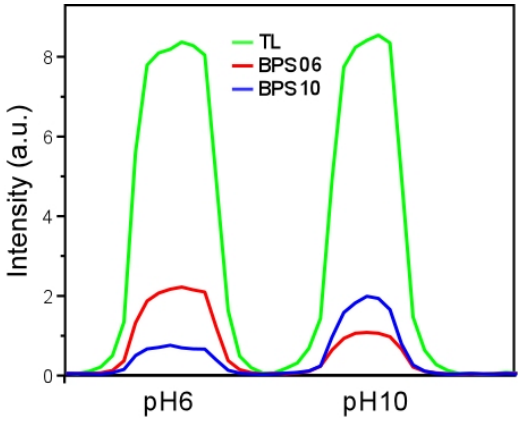

Fig. 3. Fluorescence signal obtained from two capillaries with JPTS in buffered solutions. The signal was obtained after excitation with TL and shaped pulses.

In Fig. 4, we plot the signal obtained from the setup shown in Fig. 1 with and without scattering tissue. The signal from TL pulses was multiplied by -1 and plotted as a function of position. The peak height obtained from both acidic and basic solutions is essentially the same. The small variations are caused by small shifts in the position of the focal plane. The capillary containing pure water gives no signal as expected. The difference signal obtained from the two-photon fluorescence signals (optimized for acid pH minus optimized for alkaline pH) obtained using the two masks is shown in Fig. 4 as red circles. For this measurement the signal obtained for pH 10 samples was normalized to one. Notice that the contrast, a measure of efficiency of coherent control, is quite high. In the absence of tissue the contrast ratio is 10:1. More importantly the contrast is found to survive the presence of ~ 1 mm of tissue. The contrast obtained (6:1) has been essentially maintained, although the overall signal decreases exponentially with the tissue thickness.

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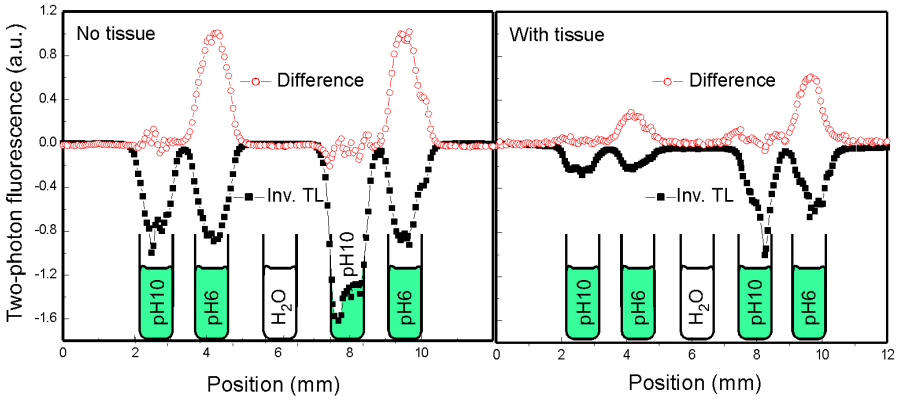

Fig. 4. Experimental results obtained with TL (black dots) pulses and difference plot obtained from the shaped laser pulses (red circles). Notice that shaped laser pulses are capable of selective excitation even when the laser transmits through scattering tissue

4. Discussion

The goal of this research was to establish if phase-shaped pulse strategies designed to control the excitation and chemistry of molecules survive transmission of the laser through scattering tissue. The results of our experiment indicate that the ballistic photons, which are the ones responsible for the two-photon fluorescence detected, retain the shaped pulse characteristics required for selective excitation. We can confirm this statement by experimentally measuring the SHG spectrum of the shaped pulses before and after transmission through biological tissue. In Fig. 5 we show the SHG spectrum of TL pulses and the SHG spectrum of shaped (BPS06) pulses with and without tissue. The result obtained with tissue gave a much weaker signal that we multiplied by a factor of 20 for the illustration. We note that the SHG spectrum of the shaped pulses suffered little or no change other than attenuation. SHG and two-photon excitation are achieved only by the coherent ballistic photons. This is why selective two-photon excitation is possible through the tissue.

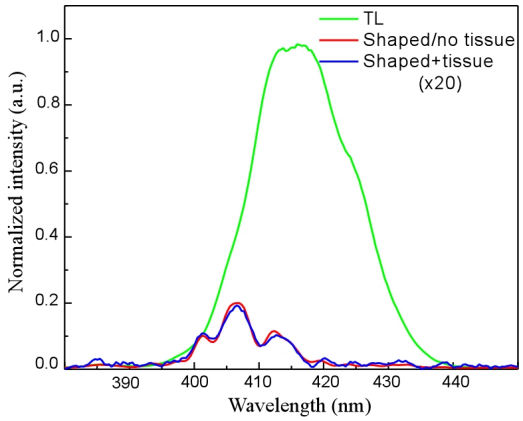

Fig. 5. SHG spectrum for TL and for shaped pulses (BPS06) in the presence and absence of biological tissue (intensity multiplied x20).

Based on these results, we can now conclude that coherent control methods can be used to improve biomedical applications of laser technology that presently use TL femtosecond pulses. In particular, we believe that optical coherence tomography, PDT, and functional imaging stand to gain the most from our result because coherent control should be able to provide better contrast and allow the use of more intense laser pulses while damage to healthy

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tissue is minimized by intrapulse interference. This method is useful for depths up to 3 mm. Delivery of the beam to internal organs could be achieved using optical fibers. For some imaging techniques it isn't possible to collect fluorescent light in the forward direction, as done here, and one most collect fluorescent photons in the backward direction (epi-fluorescence). The method used here could be implemented in that modality, we are presently setting up an inverted microscope for this purpose. Our group is actively seeking collaborations to bring these experiments to actual biomedical applications.

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