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Coherent control improves biomedical imaging with ultrashort shaped pulses

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Abstract

Although ultrashort pulses are advantageous for multiphoton excitation microscopy, they can be difficult to manipulate and may cause increased sample damage when applied to biological tissue. Here we present a method based on coherent control that corrects phase distortions introduced by high numerical aperture (NA) microscope objectives, thereby achieving the full potential of ultrashort pulses. A number of useful phase functions are recommended to gain selectivity that is similar to that which can be achieved by tuning a longer laser pulse; however this one involves no moving parts and maintains perfect optimization. This capability is used to demonstrate functional imaging by selective two-photon excitation of a pH-sensitive chromophore. Finally, we show that phase functions can also be introduced to minimize multiphoton excitation damage, while maintaining a high efficiency of two-photon excitation.

Keywords: Coherent control; Phase modulation; Shaped pulses; Femtosecond; Two-photon imaging; Three-photon

1. Introduction

Since the 1990s, imaging modalities, such as two-photon microscopy, based on nonlinear optical excitation have shown great promise [1–3]. Since nonlinear excitation scales linearly (for two-photon) or quadratically (for three-photon) with the inverse of the pulse duration, the availability of pico and femtosecond lasers has accelerated the development of multiphoton microscopy. Femtosecond lasers, especially, have been promising excitation sources for nonlinear optical imaging because of their high peak power and greater penetration ability when compared to lasers required for linear excitation. However, the expectation that a factor of ten reduction in pulse duration would lead to one order of magnitude greater signal for two-photon excitation in biological tissue was not realized, and shorter pulses were actually observed to cause greater laser induced damage [4]. This has been explained by the fact that DNA has a large absorption cross section at 260 nm, a wavelength region which can be significantly affected by three-photon excitation from a Ti:Sapphire femtosecond laser, the most common excitation source. The most frequent pulse duration used for two-photon microscopy today, therefore, is not at the lower limit of the source capability. Instead, a compromise between excitation efficiency and damage prevention is realized at ~80–100 fs.

Not all pulses with spectral bandwidths of about 20 nm are the same, unless they are transform limited (TL). A TL pulse is one whose pulse duration satisfies the energy-time uncertainty relationship $\Delta\nu\Delta\tau = 0.44$, a relation derived for pulses with a Gaussian spectrum with bandwidth $\Delta\nu$, and pulse duration $\Delta\tau$. Unfortunately, no commercial laser source produces TL pulses. Deviations from TL are usually given as a time-bandwidth product (TBP), where TL pulses have a TBP = 1. This value increases as the deviations increase. Typically femtosecond lasers have a TBP ~ 1.5. Deviations from TL cause a decrease in nonlinear optical excitation efficiency. A laser pulse with TPB = 1.5 yields 33% less two-photon and 56% less three-photon signal than a TL pulse. What is not widely appreciated is that these deviations may be strongly wavelength dependent. Therefore, similar laser sources could see different relative intensities when exciting a number of chromophores. This is especially important when the laser wavelength is tuned, and this severely impacts the quantitative prospects of multiphoton microscopy.

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The nonlinear properties of a pulse are very sensitive to spectral phase distortions that are either inherent to the laser system or are introduced by lenses, optical fibers, mirrors, or microscope objectives. Eliminating these distortions is especially critical in cases where reproducibility is an important criterion for femtosecond laser applicability. These distortions need to be corrected to ensure the fast progress of femtosecond laser excitation in biomedical imaging applications. The method presented here, multiphoton intrapulse interference phase scan (MIIPS) [5–7], not only characterizes pulses, but corrects spectral phase distortions and delivers accurate and reproducible phase information to a sample. MIIPS is a single beam technique that takes advantage of the effects of phase modulation on nonlinear optical processes to analytically acquire the spectral phase of a pulse. Spectral phase compensation through MIIPS has allowed us to perform reproducible functional imaging through scattering biological tissue. In addition, it has increased its usefulness to a wide range of areas [8] including chemical and molecular identification [9,10], multiphoton microscopy [11] and chemical microenvironment probing [6].

In this article we present a method based on coherent control that has been used to correct phase distortions in the pulse, including the significant phase distortions introduced by high NA microscope objectives, rendering TL pulses at the sample. We describe a new approach to attain excitation wavelength tunability based on phase shaping that achieves optimum efficiency, quantitative wavelength tunability, and reduces photodamage. This method has no moving parts and can even be used for imaging through thick scattering biological tissues. Finally, we demonstrate experimentally the enhancements possible through phase correction and phase control.

We focus on the sensitivity of nonlinear imaging methods to the spectral phase of the pulses and we show how controlling this phase can lead to significant improvements and new possibilities in biomedical imaging.

2. Experimental

All experiments were carried out with a 250 mW, 97 MHz Ti:Sapphire oscillator operational for sub-10 fs pulse delivery (110 nm full width at half maximum, FWHM). The pulses were shaped by introducing phase functions into the spatial light modulator (SLM) of a pulse shaper [12].

2.1. Spectral phase distortion and MIIPS

Spectral phase characterization, correction of unwanted phase distortions and the introduction of accurate phase functions were carried out with a MIIPS [5–7] setup (Biophotonic Solutions Inc.). To test the viability of pulse compensation on imaging, a mouse kidney section (FluoCells® prepared slide #3 mouse kidney section with Alexa Fluor® 488 WGA, Alexa Fluor® 568 phalloidin, DAPI) was imaged with uncompensated and with compensated TL pulses.

2.2. Tunability and selective excitation

For these experiments a sinusoidal phase function with a period corresponding to the inverse pulse duration of the laser was used to shape the pulses. Alternatively, a binary phase function, where the phase values were limited to 0 and π retardation, was used. In both cases, the point of inversion (or reflection) symmetry is where maximum two-photon excitation is expected. The laser was then focused on a thin (0.01 mm) beta-barium borate second harmonic crystal and the resulting frequency doubled spectrum was analyzed in a compact fiber-optic coupled spectrometer.

2.3. Functional imaging

Selective excitation was carried out on 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS), a pH-sensitive probe which has applications in the biological field, including monitoring intra-organelle pH in endosomal and lysosomal pathways [13]. An acidic solution of HPTS was placed in three capillary tubes, which were then immersed in a 2-mm cuvette filled with an alkaline solution of the same dye. Selective imaging of the capillary tubes required laser pulses designed to selectively excite HPTS either in acidic or alkaline solution. To achieve these shaped pulses, the unwanted phase distortions were first corrected, using MIIPS, as described above. Binary phase functions [14] were then applied with the SLM. The design of the phase functions was based on the two-photon cross section of HPTS in acidic and alkaline environments, the spectrum of the fundamental laser pulse, and the known dependence of two-photon excitation on spectral phase [15,16] and in particular to binary phase functions [14]. The laser, attenuated to 1 nJ/pulse and centered near 820 nm, was focused on the capillaries by a 20×/0.45 NA objective (Nikon Plan Fluor, extended long working distance). The two-dimensional (6 mm × 8 mm) images were obtained by scanning the sample at the focal plane of the laser; the resulting fluorescence was recorded by a spectrometer with detection wavelength set at 515 nm (24 nm spectral resolution) and averaged point by point with a lock-in amplifier.

To demonstrate the viability of selective excitation for biomedical imaging, a 500 μm slice of scattering biological (chicken breast) tissue was placed in front of the capillary tubes in the imaging setup described above. The same binary functions that were used for the selective imaging experiments generated localized two-photon excitation of HPTS at specific frequencies. Fluorescence from the excited HPTS solutions was collected from the back of the sample.

2.4. Reduced multiphoton induced damage in two-photon microscopy

The efficacy of phase modulation for maximizing two-photon excitation while minimizing three-photon transitions was demonstrated by imaging a 30 μm × 30 μm section of a slide containing the chromophores HPTS and L-tryptophan mixed with an aqueous polyvinyl alcohol (4%) solution on glass cover slips. The laser was brought in the rear of a Nikon