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Contrast improvement by using tailored laser pulses to circumvent undesired excitations

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ABSTRACT

We report on fluorescence contrast improvement by using phase, amplitude, and polarization shaped laser pulses. The measurements were conducted by applying phase functions at different spectral amplitudes for excitations of dyes and agree very well with calculations. In particular, undesired one-photon excitations are circumvented with phase and amplitude tailored pulses for two-photon transition. This is realized by cutting out the laser spectrum at the wavelength of the one-photon process while utilizing an antisymmetric phase function that allows for constructive interference of the remaining outer spectral contributions for two-photon absorption. Moreover, polarization enhanced contrast between dyes is demonstrated where the two-photon dye is predominantly excited in one polarization direction and simultaneously the one-photon dye in the other polarization direction. The presented methods of shaping ultrashort laser pulses have a high potential for imaging applications.

1. Introduction

In recent years fluorescent dyes were used as markers for imaging applications where ultrashort laser pulses were employed to distinguish between certain structures in biological samples. A large contrast is favorable in this regard to receive a clear microscopic image. In this context, the technique of laser pulse shaping provides a powerful tool to tailor the pulses such that two fluorescence dyes can be selectively excited [1,2]. Laser pulse shaping for control of photo-induced molecular processes has attained considerable success since it enables to drive the induced processes at a maximum yield along desired paths [3,4]. Moreover, a parametric subpulse encoding was developed [5], where physically intuitive pulse parameters like chirps and polarization states can be controlled which opens new perspectives of utilizing the light field in the pulse modulation. Pulse shaping techniques were already employed in life sciences in order to investigate biologically relevant systems. Here, laser pulse shaping is often applied to multiphoton excitation where intrapulse interference becomes relevant [6]. This enables to exploit interference effects in multiphoton excited fluorescence spectroscopy [7,8] and allows for three-dimensional imaging by multiphoton microscopy [9,10]. Moreover, this permits to steer molecular processes by utilizing these pulses for inducing specific multiphoton processes in molecular systems. However, in many cases the excitation spectra of the different substances are close to each other or even overlap which impedes a selective excitation. Furthermore, perturbing spectral features may hamper the intended excitations of the examined species. Methods that allow to bypass these unwanted transitions would be desirable in order to improve the received contrast.

In this contribution phase, amplitude, and polarization pulse shaping methods are described to control different excitation processes. The tailored laser pulses cause selective multiphoton induced fluorescence of dye mixtures. Special scans of frequency-

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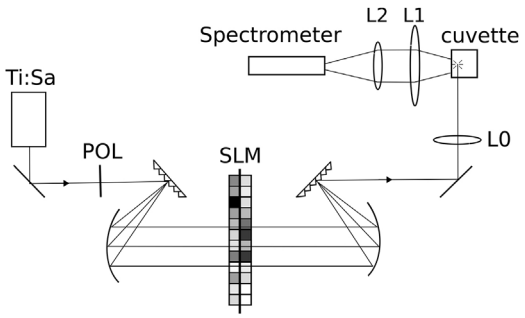

Fig. 1. Experimental setup where the laser (Ti:Sa), the spatial light modulator (SLM), the lenses (L0,L1,L2), the quartz cuvette, and the spectrometer are depicted schematically.

shifted antisymmetric phase functions will be employed to control the multiphoton excitation fluorescence [11]. The efficiency at which phase and amplitude modulated pulses with low amplitude ranges enhance imaging contrast is recorded, and the results are compared to calculations. Additionally, it will be demonstrated how a two-photon excitation can be tailored to bypass a simulated perturbing one-photon transition located in the same spectral region. In the last part, another way of circumvention by going over to the perpendicular polarization direction is realized by polarization shaping in order to increase the contrast between different dyes, whereby the phase-tailored two-photon excitation is addressed in one polarization direction and the one-photon transition is selectively excited in the other polarization direction. The presented method of phase, amplitude, and polarization pulse shaping on multiphoton excitations will be favorable regarding new biophotonic applications in endoscopy and microscopy.

2. Experimental setup

The experimental setup is schematically depicted in Fig. 1. The light source used is a titanium sapphire laser (Femtosource Compact, Femtolasers) pumped by a frequency-doubled Nd:YVO_4 laser (Verdi V, Coherent, Inc.). The average power output of the laser system is about 350 mW with a repetition rate of 75 MHz. The central wavelength is 802 nm with a bandwidth of 87 nm. The initial pulses of the oscillator are directed through a pulse shaper including a spatial light modulator (SLM 640, Cambridge Research Instruments). This setup is capable of simultaneously and independently modulating phase and amplitude of the laser pulses when a polarizer is placed behind the modulator. Phase and polarization pulse shaping is feasible by removing the polarizer. After the pulse shaper the laser beam has an average power of 11 mW. The beam is then focused into a quartz cuvette filled with the dye solution to be examined. The dyes rhodamine B (*rhoB*) and coumarin 102 (*c*102) were used for two-photon fluorescence excitation due to their high quantum yields and low fluorescence lifetimes [12,13]. The time between two consecutive pulses is roughly 13 ns which is well beyond the fluorescence lifetime of both coumarin 102 (6.5 ns [14]) and rhodamine B (3 ns [12]). The fluorescence maximum of rhodamine B is at 589 nm and the coumarin 102 fluorescence peaks at about 470 nm, hence they are well separated for selective detection by an Ocean Optics fiber spectrometer. The infrared dye ADS830AT (American Dye Source, Inc.) is utilized in the measurements involving polarization pulse shaping. The dyes were solvated in ethanol for the primary measurements, and in glycerol for the polarization shaping experiments.

The laser focus was adjusted to lie just behind the glass wall where the beam enters the cell and very close to the glass wall at the side in order to minimize self-absorption and to acquire the maximal signal. The fluorescent light is collected by two lenses and focused into the detection unit. The fluorescence was either measured with a photomultiplier tube (PMT) or when the spectral intensity was of interest with a spectrometer connected via a fiber. In order to compress the pulses an analytical optimization method, called phase resolved interferometric spectral modulation (PRISM) [15], was employed which finds the phase required for a transform-limited pulse.

3. Results

3.1. Phase scans with amplitude shaped pulses employed for two-photon excitation of different dyes

The pulse shaping experiments for two-photon excitation were conducted on the two dyes rhodamine B and coumarin 102 dissolved in ethanol. After passing the pulse shaper the laser beam is focused into the cuvette filled with the dye solvated in ethanol. In order to find a transform-limited pulse with a flat phase at the interaction region a PRISM optimization was performed. The aquired phase compensation is used as an offset in the following experiments. This yields a precise phase control which is important for the phase-sensitive measurements. In a preliminary experiment the two-photon absorption curve for rhodamine B was measured by shifting a Gaussian of 10 nm width, generated by amplitude shaping, over the whole spectrum and recording the fluorescence signal. By dividing the fluorescence signal by the square of the intensity we received the relative absorption cross-section of the dye in the accessible wavelength range. For coumarin 102 the two-photon absorption curve measured in [16] was employed for calculations.

For the phase sensitive measurements cubic phase functions $\phi = \frac{b_3}{6}(\omega - \omega_0)^3$ with a third order phase scaling factor b_3 and