

EPJ manuscript No.
(will be inserted by the editor)

Phase sensitive pulse shaping for molecule selective three-photon excitation

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Received: date / Revised version: date

Abstract In this paper we present a method for selective three-photon excitation of the two dyes, p-Terphenyl (PTP) and BM-Terphenyl (BMT), in solution by using shaped pulses. A good agreement between experiment and theoretical simulation is obtained. With this method it is possible to achieve a considerable change of the fluorescence contrast between the two dyes which is relevant for imaging applications of biological molecules.

PACS. 82.50.Pt Multiphoton processes – 82.53.Kp Coherent spectroscopy of atoms and molecules – 87.64.kv Fluorescence

1 Introduction

Selective excitation is of great interest in the area of multiphoton fluorescence microscopy. It can be used in two-photon (2-p) microscopy to increase the contrast between different fluorophores [1] [2] [3] and to suppress unwanted three-photon absorption [4]. In this investigation we demonstrate the selective three-photon (3-p) excitation of the two fluorescence dyes p-Terphenyl (PTP) and BM-Terphenyl (BMT). The main advantages of 3-p microscopy is the achievement of higher spatial resolution and larger imaging depth than in 2-p microscopy. This was demonstrated

by imaging subcortical structures in intact mouse brain in vivo [5]. 3-p excitation is for example relevant to excite the fluorescence of the amino acids typtophan and tyrosine, which do not absorb the second harmonic but the third harmonic of a Ti:sapphire-laser [6].

2 Theory

Molecule selective excitation can be achieved by coherent control of the three-photon excitation with shaped pulses, while a transform-limited pulse still results in the highest fluorescence signal. The effective field driving a mul-

tiphoton transition is the result of interference between spectral components of the pulse field. For three-photon excitations in absence of intermediate resonances the effective field of an ultrashort pulse with an electric field spectrum of $E(\omega) = |E(\omega)|e^{i\varphi(\omega)}$ is given by

$$E^{(3)}(\Delta) \propto \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} |E(\Omega_1)| |E(\Omega_2)| |E(\Delta - \Omega_1 - \Omega_2)| e^{i[\varphi(\Omega_1) + \varphi(\Omega_2) + \varphi(\Delta - \Omega_1 - \Omega_2)]} d\Omega_1 d\Omega_2, \quad (1)$$

where each photon is spectrally detuned by Ω_1 , Ω_2 or $(-\Omega_1 - \Omega_2)$ and Δ is the detuning from the third-order multiple of the carrier frequency of the pulse ($\omega - 3\omega_0$) [4][7][8][9]. For broad three-photon absorption as typically observed for laser dyes the fluorescence intensity I_{dye} is proportional to

$$I_{\text{dye}} \sim \int_{-\infty}^{\infty} |E^{(3)}(\Delta)|^2 \text{TPA}_{\text{dye}}(\omega) d\omega \quad (2)$$

where $\text{TPA}_{\text{dye}}(\omega)$ is the three-photon absorbance of the fluorescence dye and $|E^{(3)}(\Delta)|^2$ is the effective three photon spectrum. It is therefore possible to change the ratio of the intensities of two dyes by manipulating the phase $\varphi(\omega)$ to get constructive interference at low and destructive interference at high frequencies and vice versa. That is if one dye has a higher absorbance for low frequencies and the other has it for high frequencies. Examples for the effective three photon spectra of shaped pulses are shown in Fig. 1. This process is also known as multiphoton intra-pulse interference (MII) and has been demonstrated under sharp resonance condition as transitions in isolated atoms and the second harmonic generation in a thick BBO crystal and for broad transitions like the multiphotonic excitations of dyes [4][7][8][9][10][11].

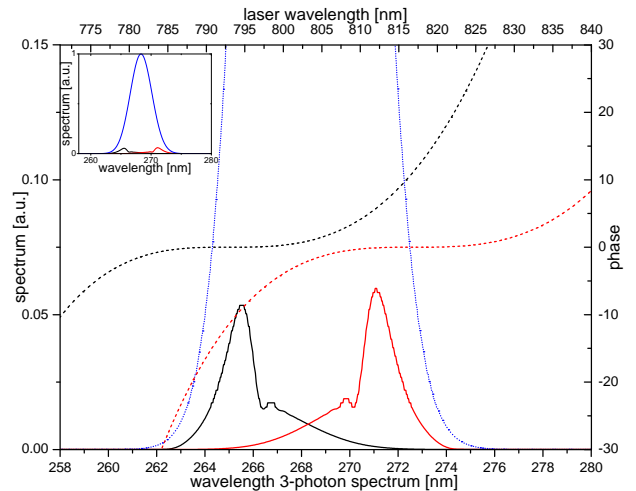


Figure 1. Effective three-photon spectrum (solid lines) and phase (dashed lines) of pulses with a cubic phase function of the form given in equation 3, for $b = 2 \times 10^5 \text{ fs}^3$, $\lambda_0 = 793\text{nm}$ (black) and $\lambda_0 = 817\text{nm}$ (red). The intensities of these spectra are lower compared to the three-photon spectrum of the transform-limited pulse (blue dotted), as can be seen in the inset on the left. The maximum of the transform-limited pulse spectrum is normalized to 1.

3 Experimental Setup

The experimental setup is shown in Figure 2. The source of the laser pulses is a regenerative amplifier (RegA 9050, Coherent) with a repetition rate of 286 kHz, a central wavelength of 805 nm and a FWHM of 22 nm. The pulses are shaped by a spatial light modulator (SLM-640, Cambridge Research Instruments) in a linear $4f$ -setup, consisting of two diffraction gratings with 1200 lines/mm and two cylindrical lenses of 250 mm focal length, which results in a resolution of 0.27 nm/pixel. To enable amplitude shaping, a polariser (POL) is placed after the SLM. The shaped pulses with energies up to 280 nJ are focused with a lens ($f = 300 \text{ mm}$) into a cuvette filled with the dis-

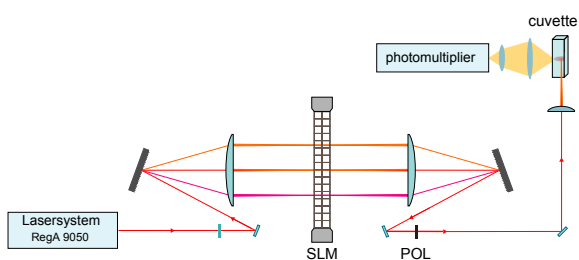


Figure 2. Scheme of the experimental setup. The pulses of the fs-Ti:Sa-laser were shaped in a 4-f-setup for simultaneous phase and amplitude shaping. The shaped pulses were used to excite the dye-solution in the cuvette. The fluorescence of the dyes is detected by a photomultiplier tube in dependence of the pulse shape.

solved dye. The fluorescence-signal of the dyes is collected using two lenses focusing the light into a photomultiplier tube (PMT, R943-02, Hamamatsu). Two colored glass filters (BG 39 and UG 11) are placed in front of the PMT to absorb scattered light in the visible as well as in the IR range. The offset phase to compensate for the chirp of the optical elements was generated by optimising the fluorescence signal with an algorithm called PRISM [12], since the transform-limited pulse results in the largest signal. This phase was added to all phase functions written on the SLM. The selective excitation is demonstrated using solutions of p-Terphenyl (PTP) and BM-Terphenyl (BMT) in ethanol. Both dyes are aromatic hydrocarbons consisting of a linear chain of three benzene rings. They were used to realize short wavelengths dye lasers with wavelength down to 321.8 nm (PTP) and 311.2 nm (BMT) respectively [13] [14]. BMT and PTP were chosen because they absorb around 268.3 nm, which is the third harmonic of the spectral pulse maximum. While the slope of the one-

photon absorption curve of PTP is positive at this wavelength the slope of the BMT curve is negative (Fig. 3). If the three-photon absorption curves are similar to the one-photon curves selective excitation should be possible. Since the selection rules for three photons excitation allow all excitations which are possible with one photon excitation such a similarity can be assumed. The concentration of dyes in the solutions was 3.5 mM for PTP and 5 mM for BMT, respectively.

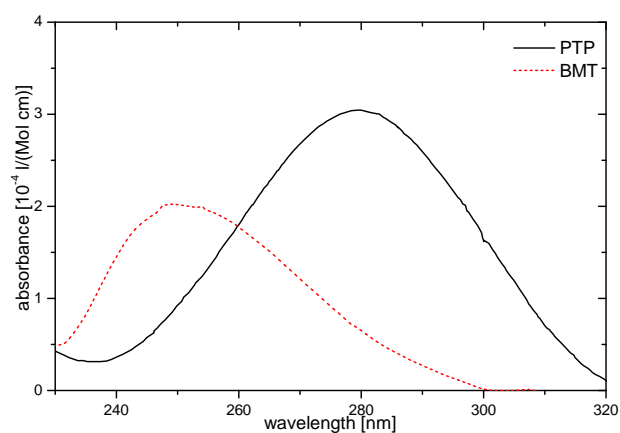


Figure 3. One-photon absorption curves of PTP (solid line) and BMT (dashed line), taken from [15].

4 Results

The fluorescence intensity ratio of PTP and BMT was recorded, to examine if selective excitation is possible. This is done by using amplitude shaping to cut out gauss spectra with a FWHM of 5 nm out of the laser spectrum, recording the fluorescence intensity for PTP and BMT in dependence of the central wavelength of these gauss spectra and calculating the ratio of the PTP- and BMT-intensity curves. The measured ratio (Fig. 4) increases

with longer wavelength, confirming a higher absorbance of BMT at shorter and PTP at longer wavelengths. For this measurement an additional home build transimpedance amplifier was used, because of the low fluorescence signal of the amplitude shaped pulses

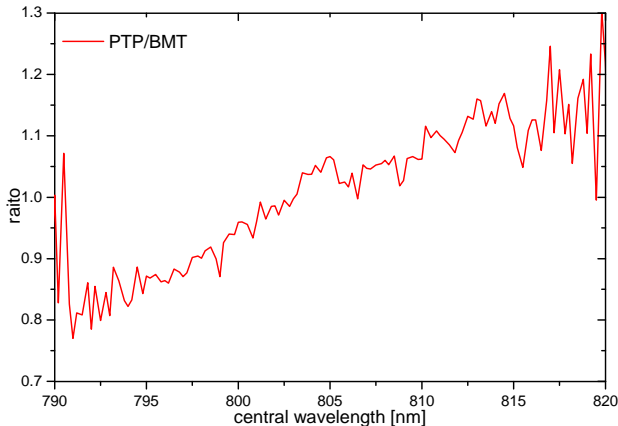


Figure 4. Ratio of the the wavelength dependent intensity curves of PTP and BMT.

For the selective excitation third-order phase functions of the form

$$\varphi(\lambda) = \frac{b}{6} \left[2\pi \left(\frac{c_0}{\lambda} - \frac{c_0}{\lambda_0} \right) \right]^3 \quad (3)$$

with the speed of light in vacuum c_0 are used. The functions are equivalent to the combined GDD-TOD phase functions, which are used for the control of two photon excitations [16][17]. Like every antisymmetric phase function their two-photon spectra have sharp maxima at $\frac{\lambda_0}{2}$ with the same intensity as the two-photon spectra of a transform-limited pulse at this wavelength. While the three photon spectra of these shaped pulses does not reach the intensity of the transform limited pulse, they still have maxima at $\frac{\lambda_0}{3}$ (Fig.1).

In the experiment third-order phase functions are the written on the modulator, for different wavelengths λ_0 . For each λ_0 the contrast c was calculated by

$$c = \frac{I_{PTP} - I_{BMT}}{I_{PTP} + I_{BMT}} \quad (4)$$

Here, I_{PTP} and I_{BMT} are the fluorescence signals of p-Terphenyl (PTP) and BM-Terphenyl (BMT) measured with the photomultiplier and normalized by the fluorescence signal of a transform-limited pulse. One of these results are displayed in Fig. 5. The measured curve is in good agreement with the simulation and a difference of $\Delta c = 0.15$ between the highest and the lowest contrast is achieved. The curve was simulated by calculating I_{dye} (Eq. 2) of BMT and PTP for all phases. Since no three-photon absorbance data for BMT and PTP is available the one-photon absorbance [15] is used as approximation. This is a valid assumption in many cases since each state that can be reached by one-photon excitation can also be reached by three-photon excitation without violating the selection rules [18][19]. Therefore the good agreement of simulation and experiment is not surprising.

5 Conclusion

In this paper the selective three-photon excitation of two fluorophores in solution is demonstrated. A change of $\Delta c = 0.15$ in the contrast of both dyes was achieved, which corresponds to a range of equal to more than a third better signal of one dye compared to the other. A significant increase of Δc can probably be reached by using a laser with higher bandwidth. In principle a similar change of

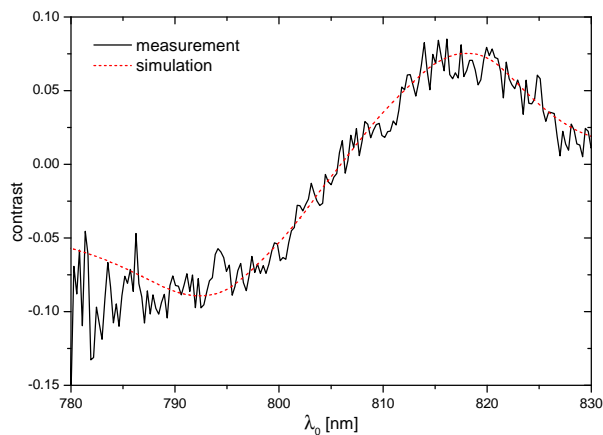


Figure 5. Contrast measurements (solid line) and simulations (dashed line) of the PTP and BMT fluorescence signal for a third-order spectral phases with $b = 2 \times 10^5 \text{ fs}^3$ and λ_0 shifted from 780 nm to 830 nm.

the contrast can be achieved by using amplitude shaping method we used to measure the fluorescence ratio in Fig.4 instead of phase shaping, but in our measurements we achieved better fluorescence signals and signal to noise ratios by phase shaping. This method could be useful for imaging techniques in biology and medicine. However since in most applications fluorescence dyes are excited in the visible spectrum this would require the use of other types of lasers than Ti:sapphire. A possible application with Ti:sapphire lasers could be the selective excitation of Tyrosin and Tryptophan.

Acknowledgments

We thank Prof. Ludger Wöste for his support.

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