Original research article

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.

MSC: 00-01; 99-00

Keywords: Ultrashort laser pulses; Laser pulse shaping; Multiphoton excitation

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-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.

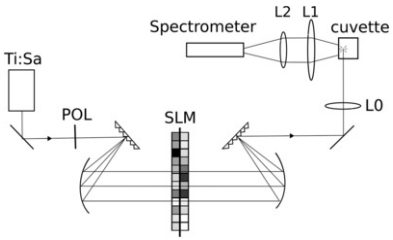

Fig. 1 Experimental setup where the laser (Ti:Sa), the spatial light modulator (SLM), the lenses (L0,L1,L2), the quartz cuvette, and the spectrometer are depicted schematically.

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 different center frequencies ω_0 were written on the modulator. The fluorescence signal from the photomultiplier was recorded while shifting the center ω_0 of the phase function. The scaling factor was set to $4 \cdot 10^4$ fs^3 and the center of the chirp was shifted in steps of 1 nm between 750 and 900 nm to cover the whole spectrum. The measurements were randomized in order to average out power fluctuations of the oscillator or bleaching of the dyes. Each measurement was repeated at least 5 times and then averaged over all scans. Particularly the influence of different amplitude functions on the fluorescence of rhodamine B and coumarin 102 was investigated. The initial spectral shape was modified by introducing regions with almost zero spectral amplitude, as shown in Fig~~. 2 and Fig. 3~~s. 2 and 3. The intensity in these regions did not go down to zero but to below 6% due to some light leaking through the shaper that is not absorbed by the polarizer.

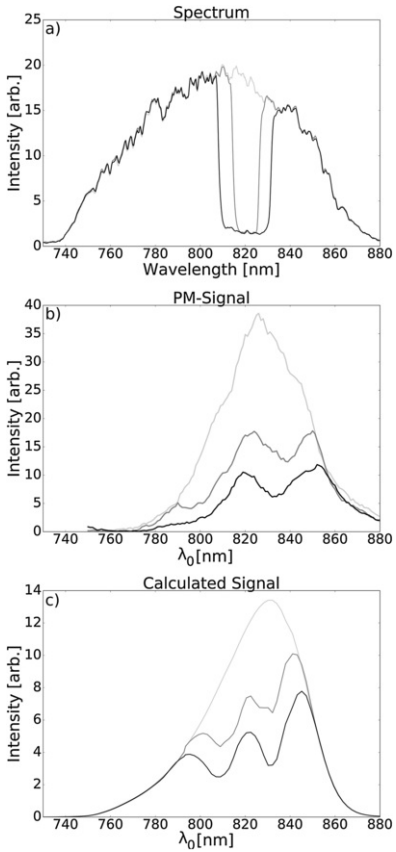

Fig. 2 Amplitude and phase modulated measurements of the two-photon fluorescence on rhodamine B. Spectra of measurements with almost zero transmission windows at widths of 6 nm (dark grey) and 12 nm (black) centered around 820 nm, and without low transmission window (light grey), are depicted in (a). The measured phase scan fluorescence signals with different window sizes of almost zero transmission are depicted in (b) for a scaling factor of $b_3 = 4 \cdot 10^4$ fs^3. The calculated two-photon signals are plotted in (c).

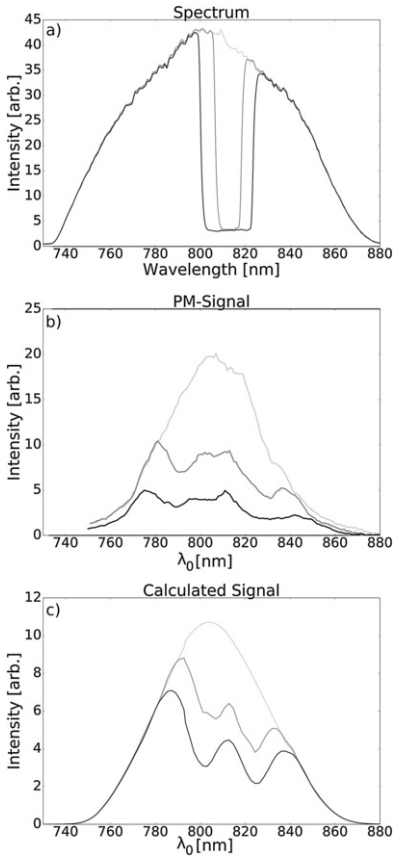

Fig. 3 Amplitude and phase modulated measurements of the two-photon fluorescence on coumarin 102. Spectra of measurements with almost zero transmission windows at widths of 6 nm (dark grey) and 12 nm (black) centered around 810 nm, and without transmission window (light grey), are shown in (a). The measured phase scan fluorescence signals with different window sizes of transmission zero are depicted in (b) for a scaling factor of $4 \cdot 10^4$ fs^3. The calculated two-photon signals are plotted in (c).

The simulation of the expected fluorescence signals was performed with a self-written script based on [7]. The measured spectra, shown in Figs. 2(a) and 3(a), and the two-photon absorption curves were included into the calculation. Specifically the cubic phase with a scaling factor of $4 \cdot 10^4$ fs^3 was applied and its center shifted from 750 nm to 900 nm. The calculated fluorescence curves shown in Fig. 2(c) and Fig. 3(c) were then obtained by integrating over the two-photon absorption.

Fig. 2 shows the two-photon fluorescence signal of rhodamine B when the center of the antisymmetric phase function is shifted along the frequency axis. The spectrum of each measurement with increasing window size is shown in the graph at the top. The acquired fluorescence signal by phase scans with different window sizes of almost zero transmission is depicted in the middle plot. Each scan was recorded with 1 nm steps, averaged over five measurements, and done with a third order phase of $4 \cdot 10^4$ fs^3. The calculated signal with the respective input values is shown at the bottom. A good agreement between experiment and theory is obtained. Fig. 3 displays the corresponding results for coumarin 102 including calculations.

The main feature is the appearing triple peak structure in the scans with the almost zero spectral amplitude ranges. It should be pointed out that in each case the maximum in the middle is present within the low transmission amplitude window, which means that there is an enhanced two-photon signal at frequencies where almost no one-photon excitation is possible. This can be explained by the constructive interference of the spectral components on both sides of the spectral window resulting in an efficient two-photon process. The constructive interference is due to the antisymmetric phase function and can be illustrated by a photon from one side of the spectrum combined with another photon from the other side of the spectrum arriving at the same time.

The different intensity ratios of the peak heights for the two dyes are caused by the differing absorption spectra. Unequal slopes of the frequency dependent absorption for the two dyes enable to influence the contrast between

the emissions of the dyes by phase shaping. For each of the performed phase scans the contrast C was calculated by $C = (I_{c102} - I_{rhoB})/(I_{c102} + I_{rhoB})$, with I_{c102} and I_{rhoB} being the integrated intensities of the corresponding spectra. Fig. 4 shows the fluorescence and contrast measurements of the two dyes for different low transmission amplitude windows around 805 nm and 815 nm, respectively. In both cases a slight increase of the contrast difference is apparent for the measurements with amplitude windows.

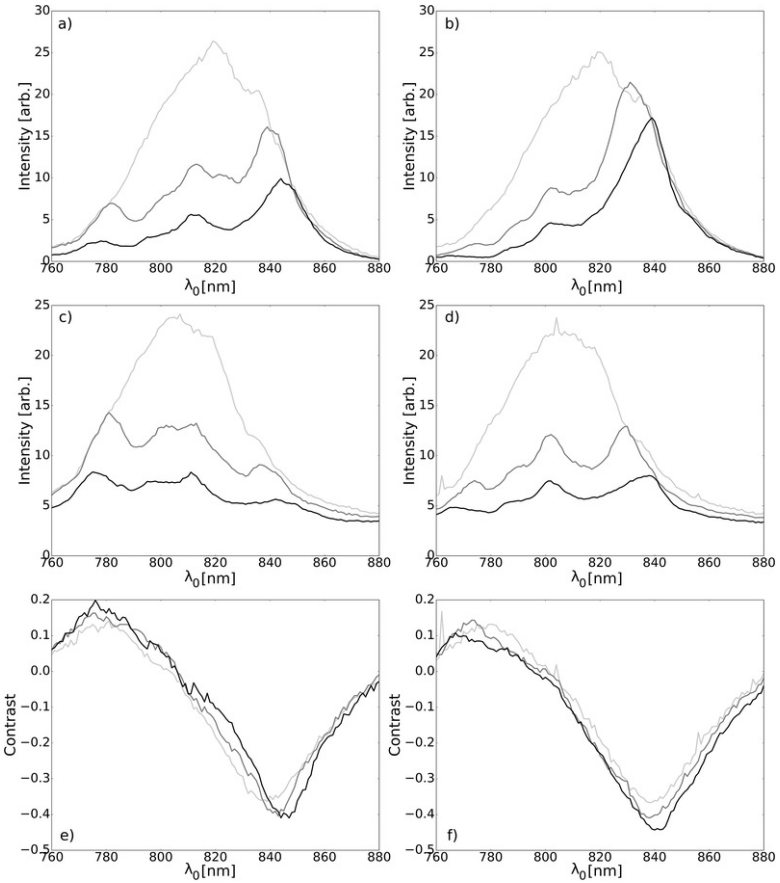

Fig. 4 Fluorescence and contrast measurements on rhodamine B and coumarin 102. For both dyes the two-photon fluorescence was measured with different regions of almost zero spectral intensities. The fluorescence measurements for rhodamine B are shown in (a) and (b) with low transmission windows centered around 805 nm (a) and 815 nm (b) with widths of 6 nm (dark grey) and 12 nm (black), and without low transmission window (light grey). They are compared with the corresponding measurements of coumarin 102, depicted in (c) and (d). The contrast between the measurements is shown in (e) with low transmission windows around 805 nm and in (f) with windows around 815 nm.

3.2 Circumvention by two-photon excitation

The striking observation that a two-photon excited fluorescence maximum is present within the amplitude window at almost zero spectral intensity can be utilized for selective excitation. The maximum originates from constructive interference of the spectra on both sides of the spectral window due to the antisymmetric phase function. This feature is used to bypass a one-photon transition within the frequency range of the amplitude window. Thereto, we performed measurements with a low transmission window for a two-photon excitation of coumarin 102 and for a one-photon excitation of the dye ADS830AT. This one-photon dye was chosen because it exhibits a relatively narrow absorption maximum close to the window position.

Fig. 5(a) shows the two-photon fluorescence of coumarin 102 (black) and ADS830AT (grey) with a 30 nm broad low transmission amplitude window around 825 nm by tuning the center wavelength λ_0 of the cubic phase function with the scaling factor $b_3 = 4 \cdot 10^4$ fs^3. This low transmission window reduces the fluorescence signal of ADS830AT to less than half of its value. Fig. 5(b) displays the obtained contrast between the two-photon fluorescence of coumarin

102 and ADS830AT where an enhanced contrast is visible at the amplitude window position. This method of bypassing an unwanted one-photon process is generally applicable and will improve the contrast for microscopic imaging.

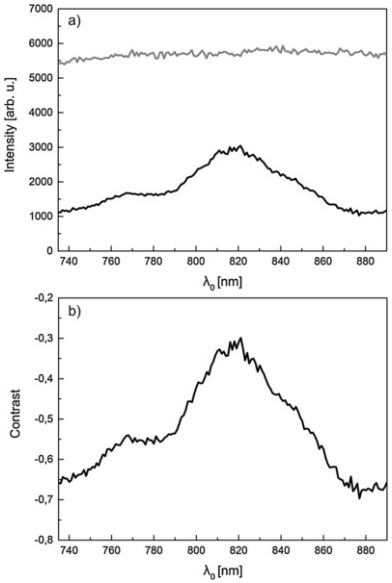

Fig. 5 (a) Two-photon fluorescence of coumarin 102 (black) and ADS830AT (grey) with a 30 nm broad low transmission amplitude window around 825 nm. The center wavelength λ_0 of the cubic phase function with the scaling factor $b_3 = 4 \cdot 10^4$ fs^3 is tuned. (b) Received contrast between the two-photon fluorescence of coumarin 102 and ADS830AT with the amplitude window. An increased contrast is obtained at the window position.

3.3 Contrast improvement by polarization shaping

If a solution is irradiated with linearly polarized light, most of the excited molecules have transition dipole moments oriented within a range of angles close to the polarization of the light. Since the polarization direction of the fluorescence photons is in most cases again parallel to the transition dipole moment, the fluorescence will then be polarized as well, if the molecules do not lose their orientation by rotation, or other effects like fluorescent resonance energy transfer. For optimally measuring the polarized fluorescence the emission was recorded after the cuvette in almost the same direction as the transmitting excitation laser beam, differing from Fig. 1. The employed dyes rhodamine B and ADS830AT were solvated in high viscous glycerol in order to maintain the molecular orientation during emission and hence the polarization direction of the fluorescence light. This feature is utilized by polarization shaping 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. Thereto, the polarizer behind the pulse shaper was removed and a spectral range of the laser pulse is polarized perpendicular to the other main spectral part. This main polarization component exhibits a cubic phase for selective two-photon excitation of rhodamine B and the perpendicular polarization component is utilized for selective one-photon excitation of ADS830AT. The latter polarization component has a quadratic phase function in order to decrease the unwanted two-photon excitation of rhodamine B.

The emission spectra for the polarization shaped laser pulses are recorded at different angles by turning the orientation of a polarizer after the cuvette. The contrasts are shown in Fig. 6 for a polarization shaped laser pulse where one polarization component has a cubic phase function $b_3 = 2 \cdot 10^2$ fs^3 with the symmetry point at 817 nm (for selective excitation of rhodamine B) and the perpendicular polarization component of the range between 800 nm and 835 nm exhibits a quadratic phase with $b_2 = 6 \cdot 10^4$ fs^2. This is compared to a pulse with the same spectral polarization ranges but a flat phase. The polarization angle of zero corresponds to the polarization direction of the cubic phase shaped component. The contrast was calculated by $C = (I_{ADS830AT} - I_{rhoB})/(I_{ADS830AT} + I_{rhoB})$, with I_{rhoB} and $I_{ADS830AT}$ being the integrated intensities of the corresponding spectra. A clearly improved contrast difference is visible for the laser pulse with the phase-shaped polarization components.

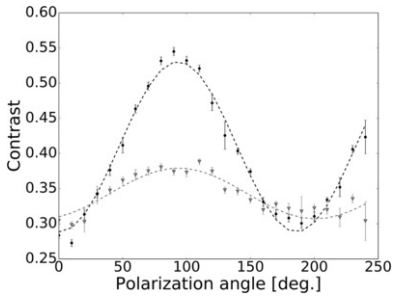

Fig. 6 Contrast between rhodamine B and ADS830AT for the phase and polarization shaped pulse (dots) depending on the rotation of a polarizer after the cuvette, and for comparison with the same spectral polarization ranges and a flat phase (triangles). The dotted curves display sinusoidal fits of the data. An enhanced contrast difference is obtained for the laser pulse with the phase-shaped polarization components.

4 Conclusions

A contrast enhancement by using phase, amplitude, and polarization shaped laser pulses was obtained for excitations of dyes in a cuvette. The experiments were performed by scanning antisymmetric phase functions for different spectral low transmission amplitude ranges. In particular, the measurements showed a distinct maximum of the two-photon yield within the spectral low transmission range which was attributed to constructive interference. A good agreement with calculations of the two-photon excitations was achieved. It was moreover feasible to bypass an unwanted one-photon excitation by employing an antisymmetric phase-shaped pulse for efficient two-photon excitation by the outer spectral contributions and removing the spectrum around the one-photon transition of the dye ADS830AT. Finally, another way of circumvention by going over to the other polarization direction is showcased by obtaining a polarization enhanced contrast between two dyes where the two-photon dye is predominantly excited in one polarization direction while the one-photon dye is simultaneously excited in the other polarization direction. The described pulse shaping methods for selective photoexcitation reveal novel perspectives for biophotonic applications.

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References

[1] T. Brixner, N.H. Damrauer, P. Niklaus and G. Gerber, Photoselective adaptive femtosecond quantum control in the liquid phase, *Nature* **414**, 2001, 57–60.

[2] M. Roth, L. Guyon, J. Roslund, V. Boutou, F. Courvoisier, J.-P. Wolf and H. Rabitz, Quantum control of tightly competitive product channels, *Phys. Rev. Lett.* **102**, 2009, 253001.

[3] R.S. Judson and H. Rabitz, Teaching lasers to control molecules, *Phys. Rev. Lett.* **68**, 1992, 1500–1503.

[4] T. Brixner and G. Gerber, Quantum control of gas-phase and liquid-phase femtochemistry, *ChemPhysChem* **4**, 2003, 418–438.

[5] F. Weise and A. Lindinger, Full parametric pulse shaping in phase, amplitude, and polarization using an effective four array modulator, *Appl. Phys. B* **101**, 2010, 79–91.

[6] D. Meshulach and Y. Silberberg, Coherent quantum control of two-photon transitions by a femtosecond laser pulse, *Nature* **396**, 1998, 239–242.

[7] V.V. Lozovoy, I. Pastirk, K.A. Walowicz and M. Dantus, Multiphoton intrapulse interference. 2. Control of two- and three-photon laser induced fluorescence with shaped pulses, *J. Chem. Phys.* **118**, 2002, 3187–3196.

[8] D. Meshulach and Y. Silberberg, Coherent quantum control of multiphoton transitions by shaped ultrashort opical pulsea, *Phys. Rev. A* **60**, 1999, 1287–1292.

[9] W. Denk, J.H. Strickler and W.W. Webb, Two-photon laser scanning fluorescence microscopy, *Science* **248**, 1990, 73–76.

[10] S.W. Perry, R. Burke and E. Brown, Two-photon and second harmonic microscopy in clinical and translational cancer research, *Ann. Biomed. Eng.* **40**, 2012, 277–291.

[11] A. Patas, G. Achazi, C. Winta and A. Lindinger, Influence of nonlinear effects on the three-photon excitation of L-Tryptophan in water using phase-shaped pulses, *J. Opt. Soc. Am. B* **31**, 2014, 2208–2213.

[12] M.J. Snare, F.E. Treloar, K.P. Ghiggino and P.J. Thistllethwaite, The photophysics of rhodamine B, *J. Photochem.* **18**, 1982, 335–346.

[13] R.F. Kubin and A.N. Fletcher, Fluorescence quantum yields of some rhodamine dyes, *J. Luminescence* **27**, 1982, 455–462.

[14] J.H. Richardson, L.L. Steinmetz, S.B. Deutscher, W.A. Bookless and W.L. Schmelzinger, Measurement of Fluorescence lifetimes of coumarin laser dyes with a mode-locked krypton ion laser, *Z. Naturforsch.* **33a**, 1978, 1592–1593.

[15] T. Wu, J. Tang, B. Hajj and M. Cui, Phase resolved interferometric spectral modulation (PRISM) for ultrafast pulse measurement and compression, *Opt. Express* **19**, 2011, 12961–12968.

[16] W.G. Fisher, E.A. Wachter, F.E. Lytle, M. Armas and C. Seaton, Source-corrected two-photon excited fluorescence measurements between 700 and 880 nm, *Appl. Spectrosc.* **52**, 1998, 536–545.