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Polarization enhanced two-photon excited fluorescence contrast by shaped laser pulses using a deformable phase plate

Polarization enhanced two-photon excited fluorescence contrast by shaped laser pulses using a deformable phase plate

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Abstract: We utilize spatially and temporally tailored laser pulses for polarization enhanced two-photon excited fluorescence contrasts of dyes. The shaped laser pulses are produced by first passing through a temporal pulse shaper and then through a novel two-dimensional spatial pulse shaper with deformable phase plates. Different spatial beam profiles are presented which demonstrate the potential of the new spatial pulse shaper. Particularly, a polarization enhanced fluorescence contrast between two dyes is reported by utilizing specific phase shaping in perpendicular polarization directions. The tailored laser pulses are further modified by the deformable phase plate and a polarization increased depth-dependent contrast is achieved. This spatial shaping for all polarization directions demonstrates the advantage of deformable phase plate spatial shapers compared to liquid crystals, where only one polarization direction can spatially be modified. The described polarization contrast method allows for three-dimensional scanning of probes and provides new perspectives for biophotonic applications.

1. Introduction

In the last years laser pulse shaping has evolved to a various tool for several research fields such as optimal control [1–4], nanooptics [5], and control of biomolecules [6]. Thereto, Fourier domain shaping with liquid crystal modulators was used resulting in temporally shaped light fields and furthermore in the modification of the light polarization which extends the pulse shaping possibilities [7–9]. Tailored laser pulses were also employed for multiphoton excited fluorescence [10], whereby intrapulse interference was utilized to selectively excite differing molecules. This approach has lead to three-dimensional imaging in multiphoton microscopy [11, 12].

Spatial modification of the laser beam profile can be conducted by using a two-dimensional modulator and corresponding focussing lenses [13]. It is carried out by liquid crystal modulators [14], or recently by a deformable phase plate modulator [15], whereby the employed phase modulations lead to desired beam shapes in the focal plane. Such spatially tailored beam profiles were employed for deep tissue focussing [16], high resolved spatial imaging [17], and microstructuring [18]. First attempts were undertaken to combine temporal and spatial shaping [19–22], because these entirely tailored pulses enable to steer photo-induced processes concurrently in time and space.

Here, simultaneously temporally and spatially polarization shaped laser pulses are employed to achieve two-photon excited fluorescence of dyes. The studies are performed with a particularly developed shaping setup first including a temporal pulse shaper and then a spatial modulator having a deformable phase plate. Initially, phase scans for different polarization conditions are presented, which indicate an increased fluorescence contrast for polarization shaped pulses compared to linear polarized pulses. Then, depth-dependent measurements are conducted by additionally using the deformable phase plate modulator. These experiments are analyzed regarding fluorescence contrasts depending on polarization states. The described pulse shaping technique exhibits novel perspectives for biophotonic applications.

2. Experimental

The experimental setup is schematically presented in Fig. 1. A Nd:YVO_4 laser (Verdi V, Coherent, Inc.) pumps a titanium sapphire laser oscillator (MIRA, Coherent Inc) having an average power of 500 mW, a pulse duration of 70 fs, and a repetition rate of 76 MHz. The central wavelength is adjusted to 800 nm with a full width at half maxium of approximately 25 nm. The generated fs-laser pulses pass a 4f pulse shaper having a computer controlled liquid crystal light modulator (SLM 640, Cambridge Research Instruments) with optical axes at ±45° to the horizontal which allows for shaping the phase and polarization of the laser light. A half-wave plate is placed after the modulator in order to rotate the light field by 45° that the light components modified by the liquid crystals are turned to horizontal and vertical direction, respectively. Such temporally shaped laser pulses are then guided through a deformable phase plate wavefront modulator (Delta 7, Phaseform GmbH) being used as a 2D spatial beam shaper in this experimental setup. The deformable phase plate has an electrode array within the active area having a diameter of 10 mm. It is an active device designed to locally change the optical path length, the geometric length times refractive index, traveled by light. This transmissive device is less than 1 mm thick and consists of a sealed liquid filled volume with a flexible polymeric membrane on one side and a rigid, transparent glass substrate on the other. The volume between the membrane and the substrate is filled with a high-refractive-index liquid. The conductive membrane is pulled toward the substrate when a voltage is applied to the electrodes. This actuation displaces the liquid, and changes the effective optical path length of light that refracts through the wavefront modulator. All polarization components can equally be shaped with this modulator.

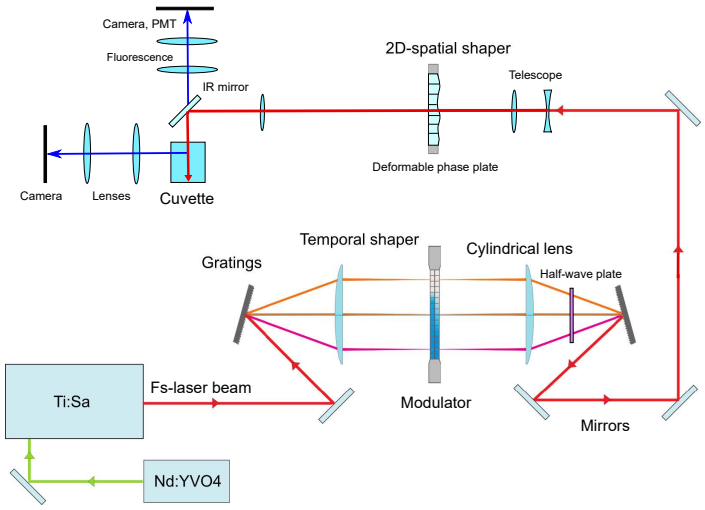

Fig. 1. Schematic experimental setup including a fs-laser (Ti:Sa), a temporal pulse shaper with gratings and a liquid crystal light modulator, and a spatial beam shaper utilizing a 2D deformable phase plate. Furthermore, a cuvette for fluorescence excitation and optical components for detection are depicted. The laser beam is first guided through the temporal pulse shaper, then through the spatial pulse shaper, and finally it is focused into a cuvette. After passing collecting lenses the fluorescence is detected in perpendicular directions, either by cameras or a photomultiplier.

The laser beam profile is enlarged by a telescope before the spatial shaper in order to use the entire active range of the deformable phase plate. A focussing lens (f = 60 mm) is located behind it to focus the laser beam into a cuvette for generating spatially modulated focal profiles.

A dielectric mirror for wavelengths around 800 nm is further placed before the cuvette in order to reflect the laser beam into the cuvette, while the fluorescence light passes the mirror (see Fig. 1). This top view geometry enables simultaneous excitation and detection on the surface normal. The cuvette is filled with the laser dyes coumarin 102 ($c102$) or coumarin 120 ($c120$) (received from Radiant Dyes) dispersed in highly viscous glycerol. The generated two-photon excited fluorescence passes an IR glass filter (BG 39) and is directed on cameras (Logitec) or a photomultiplier (Hamamatsu). The IR bandpass filter [23] is used to reduce the laser stray light in order to measure only the fluorescence signals. The fluorescence images are taken in side and top view configuration where the detectors face the side or front surface of the cuvette, respectively. Before starting the measurements, suitable phase functions are inscribed on the temporal modulator to achieve a short pulse resulting in a constant phase which is a prerequisite for the phase-controlled measurements.

3. Results

3.1. Two-photon excited fluorescence by polarization tailored pulses

The present pulse shaper setup allows for generating polarization shaped pulses with independently tailored perpendicular polarization components. This is conducted by separately inscribing the corresponding voltages on the two liquid crystal arrays of the temporal modulator which have perpendicularly arranged optical axes and leads to independent phase shaping of the two perpendicular polarization components. The half-wave plate located behind the temporal modulator turns the light field by 45° in order to orient the light components shaped by the liquid crystals to the horizontal and vertical direction, respectively. This is done to prevent the second polarization dependent grating from changing the angle between the shaped polarization components. The subsequent spatial modulator does not modify the polarization directions. The two-photon excited fluorescence for such pulses is recorded for coumarin 102 and coumarin 120, respectively.

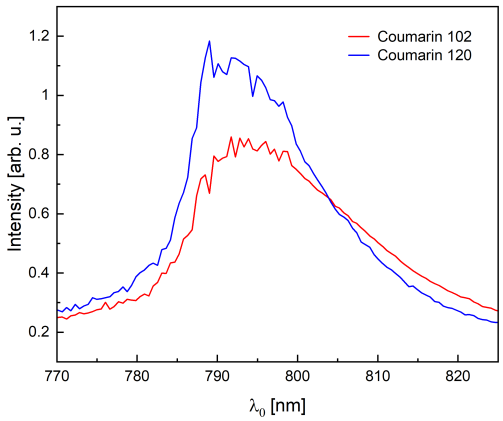

Fig. 2. Two-photon excited fluorescence signals by scanning the excitation phase center wavelength are presented for coumarin 102 (red) and coumarin 120 (blue) generated for perpendicularly polarized subpulses. Third order phase scans of the first subpulse were performed and the fluorescence was recorded behind a horizontally oriented polarizer. A red-shift of the curve for coumarin 102 compared to coumarin 120 is obtained.

The two perpendicularly oriented polarization components can further be phase-shaped to obtain selective two-photon excitations. Antisymmetric third order phase functions are used to receive spectrally narrow two-photon excited fluorescence maxima due to constructive

interference, similar as described in [10]. Phase functions $\phi(\omega) = \frac{b_3}{6}(\omega - \omega_0)^3$ with a third order phase factor b_3 and differently tuned phase center frequencies ω_0 are independently inscribed on the perpendicular polarization components of the temporal pulse shaper. The phase center value is thereby stepwise moved from lower to higher wavelengths by inscribing the corresponding voltage values on the modulator. Constructive interference close to ω_0 results in spectrally narrow two-photon maxima and enables two-photon excited fluorescence contrast enhancement.

Fig. 2 shows the two-photon excited fluorescence of coumarin 102 and coumarin 120, respectively, for a wavelength scan of $\lambda_0 = 2\pi c/\omega_0$ with a phase factor of $b_3 = 5 \cdot 10^5$ fs^3 of the first horizontally polarized subpulse. This is done in the top-view geometry, where the photomultiplier faces the cuvette wall of the laser entrance (see Fig. 1). A horizontally oriented polarizer is thereby placed in the fluorescence path. A red-shift of coumarin 102 signals compared to coumarin 120 can be observed, where a value of 1.8±0.8 nm was found for the red-shift by calculating the difference of the arithmetic means of the two curves. This can be rationalized by a higher two-photon absorption cross-section of coumarin 102 relative to coumarin 120 by proceeding to larger wavelengths. Contrast curves can be determined from the scan data.

3.2. Contrast enhancement with polarization shaped pulses

The two-photon excited fluorescence characteristics by polarization-shaped laser pulses is investigated for different polarization conditions. Thereto, a polarizer is placed at different positions and the results are compared. The fluorescence yield is influenced by antisymmetric third order phase functions inscribed on the temporal pulse shaper which lead to constructive two-photon excited fluorescence close to the antisymmetry wavelength of the phase function. The applied laser pulses consist of two perpendicularly polarized subpulses with a time delay of 400 fs. The first horizontally polarized subpulse exhibits a third order phase with a phase factor of $b_3 = 5 \cdot 10^5$ fs^3 and the antisymmetry point will be spectrally scanned. The second vertically polarized subpulse has a third order phase with a phase factor of $b_3 = 5 \cdot 10^4$ fs^3, an antisymmetry wavelength of $\lambda_0 = 790$ nm, and is kept constant during the measurement.

In Fig. 3 the center wavelength of the third order phase is scanned for the first subpulse. The contrasts $c = (I_{c102} - I_{c120})/(I_{c102} + I_{c120})$ of third order phase center wavelength scans with different polarization adjustments are presented, where I_{c102} and I_{c120} indicate the fluorescence intensities of coumarin 102 and 120, respectively. The measurements were performed with horizontally or vertically oriented polarizer in the fluorescence path and with a polarizer placed in the laser excitation path, oriented at 45° to the horizontal, which delivers a linearly polarized laser pulse with two delayed sub pulses. The recorded data allow for a comparison between polarization-shaped and linear polarized laser pulses. The maximal contrast difference of the data with vertical polarizer ($\Delta c = 0.12$) is smaller than for the linearly polarized case ($\Delta c = 0.2$), whereas the contrast difference for the horizontal polarizer is larger ($\Delta c = 0.3$). This indicates the dependency on polarization and proves that the contrast difference can be enhanced by polarization shaped pulses.

A further experiment was performed in order to explore the polarization-dependent contrast. Two perpendicularly polarized subpulses were generated, where the first exhibits a phase factor of $b_3 = 5 \cdot 10^5$ fs^3 and an antisymmetry wavelength of $\lambda_0 = 812$ nm, and the second with the same properties as explained above. The left hand inset from Fig. 3 shows a schematic 3D-image of the polarization shaped pulse. Two-photon excited fluorescence measurements were performed for both dyes, whereby the polarizer in the fluorescence path was turned in order to receive polarization-dependent data. The right hand inset of Fig. 3 displays the two-photon excited fluorescence contrast between coumarin 102 and coumarin 120 by tuning the polarizer angle relative to the horizontal. A maximum at 0° and a minimum close to 90° is obtained. This indicates that coumarin 102 is predominantly excited by the horizontally polarized subpulse and coumarin 120 by the vertically polarized subpulse. It is in agreement with the phase scan results

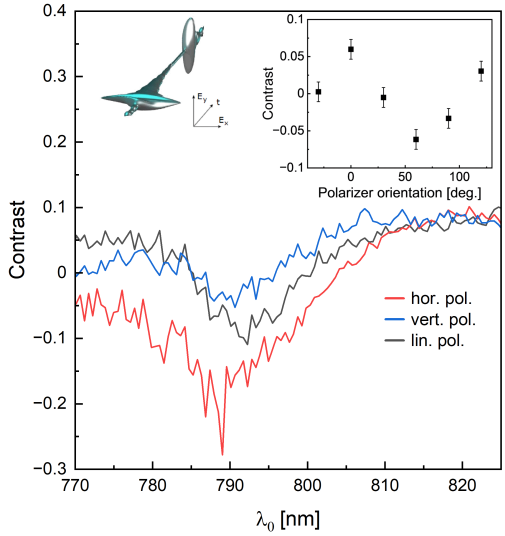

Fig. 3. Contrast curves between coumarin 102 and 120 for third order phase center wavelength scans with different polarization conditions. The measurements were conducted for polarization-tailored pulses with two subpulses, whereby a horizontally (red) or vertically (blue) oriented polarizer is located in the fluorescence path. By placing a polarizer in the laser path, contrast data of a linearly polarized laser pulse (black) were generated. A polarization dependence of the maximal contrast difference is obtained and an enhanced contrast difference for polarization-shaped pulses is received. The left hand inset shows a schematic 3D-image of the polarization shaped pulse. The right hand inset displays the two-photon excited fluorescence contrast by rotating the polarizer in the fluorescence path. A maximum at the horizontal and a minimum close to the vertical direction is observed which indicates that coumarin 102 is selectively excited by the horizontally polarized subpulse and coumarin 120 by the vertically polarized subpulse.

from Fig. 3 and shows that one dye is selectively excited in one polarization direction and the other dye in the other polarization direction. This can be regarded as a polarization enhanced two-photon excited fluorescence contrast.

3.3. Spatially shaped fluorescence structures generated by a deformable phase plate modulator

Spatially tailored laser profiles can be generated by the deformable phase plate modulator located behind the temporal pulse shaper. Computer-controlled voltage values are applied on the individual modulator elements to modify their thickness, and Zernike polynomials are used for phase control which leads to spatial modulation in the focal plane of the convex lens behind the modulator. Zernike polynomials are used in optics e.g. to describe wave front aberrations. They have a polynomial radial and an azimuthal angular part and are separated into even $Z_n^m(\rho,\phi) = R_n^m(\rho)cos(m\phi)$ and odd $Z_n^{-m}(\rho,\phi) = R_n^m(\rho)sin(m\phi)$ functions, with the radius ρ and the azimuthal angle ϕ. In the following the value multiplied with the Zernike polynomial will be called prefactor.

Fig. 4 shows the astigmatic influence of different Zernike polynomials. Solely for this experiment, a screen is placed at the position of the cuvette and photos of the beam profiles were taken. The spot can be e.g. elliptically shaped by Z_2^2 polynomials (Fig. 4 (b) and (c)) or by Z_2^{-2} polynomials (Fig. 4 (d) and (e)). It can be observed that the profiles are elliptically shaped with

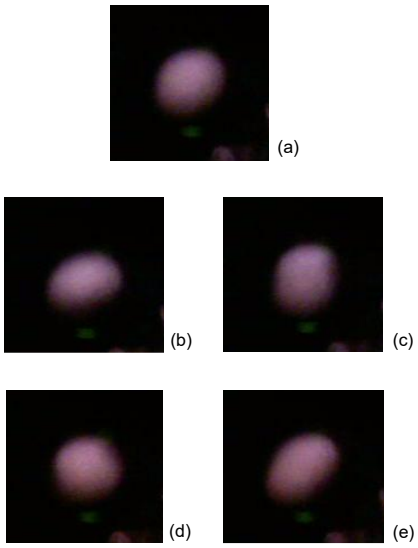

Fig. 4. Beam profiles for different Zernike polynomials. The images display the light intensities on a screen without spatial shaping (a) and with astigmatic spatial phase shaping by a Z_2^2- Zernike polynomial with the prefactors 5 (b) and -5 (c), and by a Z_2^{-2}- Zernike polynomial with the prefactors 5 (d) and -5 (e).

horizontal (b), vertical (c), +45° (d), and −45° (e) main axes which is in agreement with the expected astigmatic outcomes.

Particularly, the two-photon excited fluorescence structure can be modified by forming the beam profile. This is demonstrated by moving the focal depth position with Z_2^0 -Zernike polynomials. Fluorescence images are presented in Fig. 5 where two adjacent cuvettes are observed from the side with coumarin 102 filled in the left (rear) and coumarin 120 in the smaller right (front) cuvette. The green fluorescing focus is located in the rear cuvette by using a prefactor of -5 and the blue fluorescing focus in the front cuvette with a prefactor of 5. These measurements were conducted with short pulses having a constant spectral phase.

3.4. Depth-dependent two-photon excited fluorescence of polarization shaped pulses

A good contrast between different substances on small distances is important for fluorescence imaging. Here, this goal will be approached by simultaneous temporally and spatially tailored pulses in order to perform selective depth-dependent two-photon excitations of different dyes. Hence, a depth-dependent contrast measurement with polarization-shaped pulses is conducted. The employed pulses exhibit a first horizontally polarized subpulse with a third order phase factor of $b_3 = 5 \cdot 10^5$ fs^3 and an antisymmetry wavelength of $\lambda_0 = 812$ nm, and a 400 fs delayed second vertically polarized subpulse with a third order phase factor of $b_3 = 5 \cdot 10^4$ fs^3 and an antisymmetry wavelength of $\lambda_0 = 790$ nm. The polarized pulse focus is moved by about 2 mm (see Fig. 5) from one cuvette to the other by tuning the prefactor of the Z_2^0 -Zernike polynomial, and the fluorescence is measured on the front side. The front cuvette is filled with coumarin 120 and the rear cuvette with coumarin 102. This order is favorable because the lower frequency fluorescence from the rear cuvette will not be absorbed by the front cuvette, whereas absorption would occur the other way around.

The experiment is again conducted with a horizontally or vertically oriented polarizer in the fluorescence path and with a polarizer in the laser path, oriented at 45° to the horizontal, the

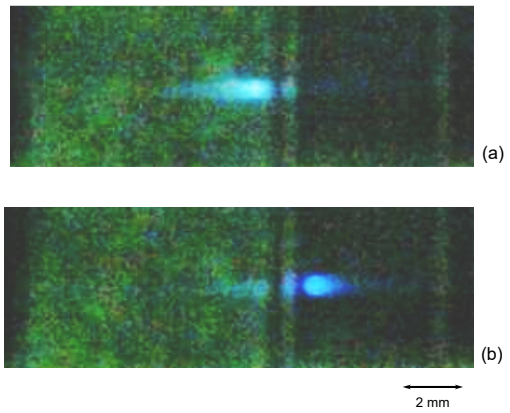

Fig. 5. Modification of the focal depth position by the Z_2^0 -Zernike polynomial. Two adjacent cuvettes are visible from the side with coumarin 102 filled in the left (rear) and coumarin 120 in the smaller right (front) cuvette. The focus is located in the rear cuvette by using a prefactor of -5 (a) and it is moved to the front cuvette by a prefactor of 5 (b).

latter leading to a linearly polarized laser pulse. The two-photon excited fluorescence data for the three different polarization conditions are shown in Fig. 6. The dip in the curves close to zero can be explained by reduced fluorescence due to the cuvette walls. At these focus positions both dyes may contribute to the signal since the focal range is partially present in both cuvettes. The lower intensity of the vertical compared the horizontal component can be explained by the reduced reflection of the second grating in the temporal modulator for vertical polarization. Particularly for the horizontally oriented polarizer, an enhanced signal ratio is obtained for the rear to the front cuvette compared to the linearly polarized pulse and a reduced ratio is observed for the vertically oriented polarizer. The corresponding contrast for the horizontal case of 0.06 is hence increased compared to the contrast of 0.03 for the linearly polarized case, and the contrast for the vertical case is decreased to -0.02. This demonstrates that the spatially dependent contrast can be enhanced by polarization shaped laser pulses. Since these polarization tailored laser pulses can well be spatially moved by the deformable phase plate modulator, three-dimensional scans of probes can be performed. The spatial modification without changing the polarization state is beneficial compared to spatial shaping by liquid crystal modulators, where only a single polarization direction can be modulated.

4. Conclusion

Spatially and temporally polarization shaped laser pulses were applied for two-photon excited fluorescence of dyes. The pulses were shaped including different polarization directions which means that they exhibit a temporal profile, where the polarization direction is changed during the pulse. These investigations were conducted by using a combination of a temporal pulse shaper and a novel deformable phase plate spatial modulator. Characteristic third order phase scans were recorded for different polarization directions. They exhibit an enhanced two-photon excited fluorescence contrast difference for polarization shaped pulses compared to linear polarized pulses. Moreover, different spatially modified beam profiles were presented. Particularly, depth-dependent two-photon excited fluorescence experiments were performed by utilizing the deformable phase plate shaper. The measurements feature an increased spatial fluorescence contrast for specific polarization-shaped pulses compared to linearly polarized pulses. The polarization-tailored laser pulses, which lead to increased contrast differences, can hence spatially

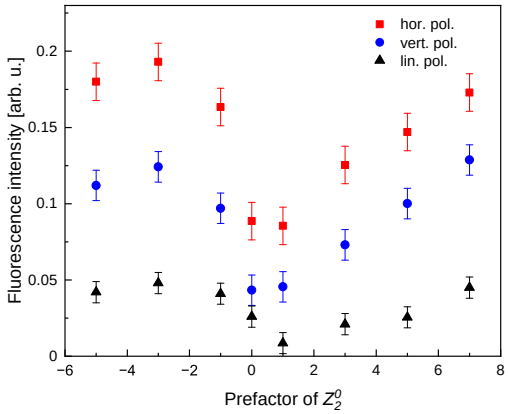

Fig. 6. Depth-dependent fluorescence for differing polarization conditions. The measurement is recorded with a horizontally (red) or vertically (blue) oriented polarizer in the fluorescence path and a polarizer in the laser path, oriented at 45° to the horizontal (black), which results in a linearly polarized pulse. The two-photon excited fluorescence is measured for the three polarization conditions. In horizontal direction an enhanced signal ratio is obtained for the rear to the front cuvette compared to the linearly polarized pulse and to the vertical direction which shows that the spatially dependent contrast can be increased for polarization-tailored pulses.

be modified by the deformable phase plate modulator. The described spatial modification for all polarization directions is favorable compared to spatial shaping by liquid crystal modulators, whereby only one polarization component can spatially be modulated. Since the deformable phase plate modulator is relatively small it can well be integrated into microscopes in order to improve the image by adaptive optics. The polarization contrast technique developed here can be employed for three-dimensional scans of biological probes and has a high potential for imaging applications.

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Disclosures. The authors declare no conflicts of interest.

Data Availability Statement. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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