Linear Dichroism and Birefringence in Polarization-Modulation Pump-Probe Spectroscopy

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Abstract. We present the refinement of the Polarization-Modulation Pump-Probe method developed recently in our group (PCCP V. 22, 18155 (2020)) for separation and quantitative determination of linear dichroism and birefringence of the probe laser beam in biologically relevant molecules. The method was used in the study of ultrafast relaxation dynamics in the excited states of NADH in aqueous solution. The probe beam birefringence contained contributions both from resonance pump beam absorption in NADH and from nonlinear coherent effect that was attributed to the stimulated Raman scattering in water, the latter manifested as a very intense and narrow peak at sub-picosecond delay times between pump and probe pulses. The probe beam dichroism contained the contribution from the resonance pump beam absorption in NADH and practically no contribution from nonlinear coherent effects. In this case the experimental signal was approximately twice smaller than that of the birefringence case. Therefore, it was suggested that the birefringence detection allows to achieve higher sensitivity for determination of the relatively long relaxation times than the linear dichroism detection.

Keywords: polarization-modulation pump-probe; linear dichroism; birefringence; NADH; Stimulated Raman Scattering.

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1 Introduction

Ultrafast pump-probe polarization spectroscopy has emerged as a powerful tool for investigation of photoinduced processes, biomedical imaging, and material science including molecular reorientation, energy transfer, and anisotropic relaxation dynamics [1–13]. Excitation of polyatomic molecules in fluid solution with polarized light can lead to transient birefringence (BR) and linear dichroism (LD) resulting in the change of polarization of the probe beam passed through a molecular sample. In both cases the experimentally measured value is usually recorded as a difference between the intensities of two orthogonal transient probe beam polarization components.

If the pump and probe laser pulses do not overlap in time the physical quantities that underlie the recorded experimental signals are the real and imaginary parts of the linear susceptibility $\chi^{(1)}$ [1, 3, 4, 11, 13]. In the field of ultrafast dynamics in transparent fluids that is widely used nowadays this phenomenon is usually named optical Kerr effect (OKE) [2, 5, 9]. If the pump and probe laser pulses overlap in time the physical quantities that underlie the recorded experimental signals are often the real and imaginary parts of the nonlinear third- order susceptibility tensor $\chi^{(3)}$ measured within the Raman-induced Kerr effect (RIKE) [6, 7, 14]. According to our recent theoretical analysis [15, 16] both effects can be described by the same light polarization dependencies and treated in a uniform way.

The advantage of the pump-probe polarization spectroscopy is its ability to provide polarization-sensitive images reflecting molecular reorientation and anisotropic relaxation processes at high spatial and sub-picosecond temporal resolution. On the other hand, a disadvantage of all pump-probe schemes is that they require the measurement of a weak differential transmission signal sitting on top of the large and

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fluctuating background given by the probe light beam. Another important source of experimental errors in the pump-probe method is the heating of a molecular sample by the pulsed laser beams that form an oscillating thermal lens that disperses the probe beam. A common way of extraction of the differential polarization signals is modulation of the pump light beam intensity with following lock-in detection at the modulation frequency [1–8]. This technique allows to decrease significantly the excess fluctuations of the probe laser beam intensity in the frequency band below the modulation frequency. However, the modulation of the pump beam intensity causes a thermal lens in the molecular sample that oscillates at the modulation frequency and gives contribution to the signal noise.

An additional effective approach for intrinsic rejection of excess laser noise is the balanced detection scheme [7]. Within this approach the orthogonal polarization components of the probe beam are separated by a polarization prism and then recorded simultaneously by two identical photodetectors and subtracted from each other by a differential integrator. However, the authors of Ref. [7] still used the pump beam intensity modulation and therefore their results were not free from the thermal lens effect.

We have recently suggested a new method that we call Polarization-Modulation Pump-Probe (POMO-PP). The main idea of our method is high-frequency modulation of the pulse train polarization in the pump beam with following balanced detection scheme of the transmitted probe beam polarization components, and recording of the differential signal at the modulation frequency using lock-in detector. The modulation of the pump beam polarization allows to keep constant the pump laser intensity in the molecular sample and thus avoid the oscillating thermal lens effect that allows for effective suppression of both laser beam noise and thermal lens effect [10, 11]. The polarization modulation was used before [9], however without the balance detection scheme, and the pump beam intensity modulation was still utilized. The combination of experimental techniques used in our method dramatically increased sensitivity and allowed to use laser pulses from a femtosecond oscillator with energies lower than a nJ for effective detection of LD in the excited state of nicotinamide-adenine-dinucleotide (NADH) molecules diluted at low concentration in various solutions [11].

In this work we further developed the POMO-PP method suggested in our papers [10, 11]. The key improvement of the method lies in the ability to independently isolate and quantify the LD and BR signals induced in the molecular sample by polarized pump pulses. The verification of the method was performed through the analysis of polarized beam propagation by means of the Müller matrix formalism [17]. The method allows to combine the detailed study of ultrafast photophysical processes and energy transfer in molecular excited states with unprecedented sensitivity of the approach developed before [11].

2 Experiment

2.1 Experimental Method

The main features of the method are as follows. A linearly polarized pump femtosecond laser pulse interacts with initially isotropic molecular sample and produces the anisotropic distribution of excited molecular axes. This anisotropy is detected by a collinearly propagated probe laser beam polarized at 45° to the direction of the pump beam polarization. The femtosecond probe pulse is delayed by the time τ with respect to the pump one. Passing through the anisotropic sample the probe beam undergoes linear dichroism and birefringence that result in the rotation of its polarization plane and in the appearance of ellipticity. The change of the polarization state of the probe pulse is detected by sequentially installed quarter wave plate and a Glan prism.

As is well known, the experimental signal recorded within such experimental schemes is usually noisy suffering mainly from the fluctuations of the probe laser beam intensity and the thermal lens effect [18, 19]. For significant reduction of the influence of both these effects the polarization of the pump beam was modulated at the frequency of 100 kHz resulting in the periodic linear polarization change from horizontal (X) to vertical (Y) positions. Also, the detection of the probe beam polarization changes was made through a balanced detection scheme that consisted of two identical photodiodes and a home-made differential integrator. The resulting differential signal, modulated at 100 kHz, was demodulated using a lock-in amplifier with a bandwidth of 3 Hz. Separation of the linear dichroism and birefringence effects was realized by changing the direction of the quarter-wave plate axis that was set at $\theta = 0$ and $\theta = \pi/4$ for detection of the probe beam linear dichroism and birefringence, respectively. The developed method allows to separate the effects of linear dichroism and birefringence of the probe beam with unprecedented detection sensitivity.

Justification of the method was carried out by calculating the change of the probe beam polarization using Müller matrix formalism [17]. The expression for the difference of the probe light orthogonal polarization component intensities $\Delta I = I_x - I_y$ after passing through the quarter wave plate and the Glan prism as a function of the quarter wave axis direction angle θ can be presented in the form:

$$\Delta I = I_{pr}(S_1 \cos^2[2\theta] - \\ - S_2 \cos[2\theta] \sin[2\theta] - S_3 \sin[2\theta]), \quad (1)$$

where S_1, S_2, S_3 are the Stokes parameters that define the light polarization before the quarter wave plate, θ is the angle of fast axis of quarter-wave plate relative to the vertical direction y, and I_{pr} is the probe beam intensity.

The Stokes parameters are defined as following Eq. (2) [20]

$$\begin{aligned} I_{pr} &= I_x + I_y, \\ S_1 &= \frac{I_x - I_y}{I_{pr}}, \\ S_2 &= \frac{I_{x\prime} - I_{y\prime}}{I_{pr}}, \\ S_3 &= \frac{I_{RC} - I_{LC}}{I_{pr}}, \end{aligned} \qquad (2)$$

where I_x and I_y are intensities of the orthogonal polarization components along x and y axes, $I_{x'}$ and $I_{y'}$ are the intensities of the orthogonal polarization components along the axes x' and y' that are rotated by the angle of 45°, and I_{RC} and I_{LC} are the intensities of right- and left-handed circular polarization components.

As can be seen in Eq. (1) if the angle θ is equal to 0 then only the Stokes parameter S_1 contributes to the detected signal, while if this angle is equal to $\pi/4$, then only the Stokes parameter S_3 contributes. These angles also satisfy the balanced detection scheme used in our experiment that yields a zero output signal when the pump beam is turned off. Therefore, at the angles $\theta = 0$ and $\theta = \pi/4$ the noise of the probe beam intensity fluctuations in the recorded signals is effectively reduced. Moreover, the modulation of the pump beam polarization at 100 kHz combined with the balance detection scheme and the following lock-in detection result in an additional significant reduction of noise from the probe laser intensity fluctuations and the thermal lens effect. As a result, as shown in this paper (see also Refs. [10, 11]) the anisotropic relaxation processes in the excited states of polyatomic molecules can be recorded by relatively low-energy (nJ) femtosecond pulses from a femtosecond oscillator without an amplifier.

3 Experimental Setup

A simplified scheme of the experimental setup is shown in Fig. 1.

A femtosecond oscillator MaiTai HP (Spectra Physics, USA) was used as a light source. A laser pulse duration was about 180 fs with a bandwidth of 8 nm and the repetition rate of 80 MHz. The laser fundamental output at 736 nm was split into two beams, one of them was directed on to the second harmonic generator (SHG) (Inspire Blue, Spectra Physics, Spain) to produce the pump beam at 368 nm. The pump beam passed through a photoelastic modulator (PEM) (PEM-100, Hinds Instruments, USA) operating at the frequency of 50 kHz. The modulation of the pump beam polarization resulted in the periodic linear polarization change from horizontal (X) to vertical (Y) at the frequency of 100 kHz. The modulated pump beam was focused on a 1 mm cuvette with solution, forming a spot of approximately 43 μm. After passing through the cuvette, the pump beam was reflected by a dichroic mirror (DM2) on a silicon photodetector (D) that controlled the beam intensity.

The second beam at the fundamental wavelength of 736 nm was directed to a motorized delay line and used as a probe beam. The probe beam passed through a delay line, was reflected from a dichroic mirror (DM1), and propagated toward the cuvette collinearly with the pump beam. The probe beam polarization plane was set at 45° to the vertical polarization plane (y) of the pump beam. It was focused into the spot of about 50 μm in the cuvette.

After the cuvette the probe beam passed through DM2, an absorption filter, and then was directed to the analyzer consisted of a quarter-wave plate ($\lambda/4$) and a Glan prism (GP). Two orthogonal polarization components of the probe beam were separated by the Glan prism and then recorded by a balanced detection scheme. The scheme consisted of two identical silicon photodiodes (Dx, Dy) (DET10A/M, ThorLabs, USA) and a home-made differential integrator (DI) with a passband of 0–4 MHz. The resulting differential signal, modulated at 100 kHz, was demodulated using a lock-in amplifier (SR844 RF, Stanford Instruments, USA) with a bandwidth of 3 Hz and subsequently recorded for computer analysis. The laser pulses energies in front of the cuvette were controlled by attenuators and set to 470 and 170 pJ for pump and probe beams, respectively.

The direction of the quarter-wave plate $\lambda/4$ axis was set at $\theta = 0$ and $\theta = \pi/4$ for detection of the probe beam linear dichroism and birefringence, respectively.

In experiments we used freshly prepared water solutions of β-Nicotinamide Adenine Dinucleotide (NADH, disodium salt, Sigma-Aldrich). The β-NADH salt was dissolved in distilled water to a concentration of 0.4 mM. This resulted in an absorption of about 14% of pump beam. This absorbance level was chosen to ensure sufficient signal while preventing effects associated with absorption heterogeneity across the 1 mm cuvette path length.

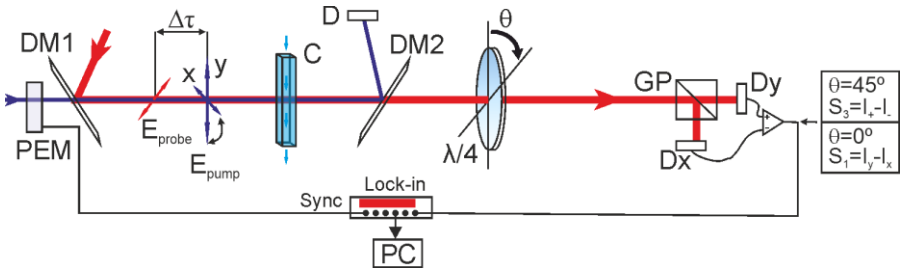

Fig. 1 Scheme of the birefringence and linear dichroism measurements; $\Delta\tau$ is a delay time between the pump and probe pulses; PEM is a photoelastic modulator; DM1, DM2 are dichroic mirrors; GP is a Glan prism; D, D_x, D_y are detectors; C is a cuvette with solution; θ is an angle of quarter-wave plate fast axis relative to the vertical axis y.

4 Results and Discussion

The BR and LD signals of the probe laser beam recorded in NADH in aqueous solution are shown in Figs. 2 and 3, respectively, as a function of the delay time τ between the pump and probe pulses.

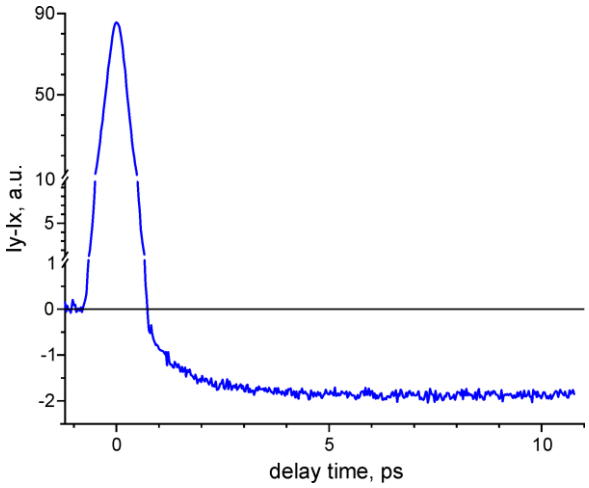

Fig. 2 BR signal of 0.4 mM NADH up to 11 ps at 45°.

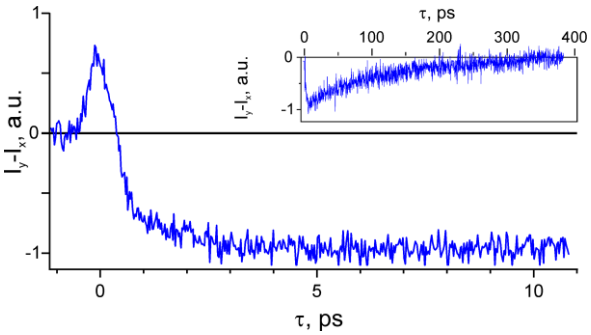

Fig. 3 Linear dichroism signal of 0.4 mM NADH up to 11 ps at 0°. The inset shows the long signal up to 400 ps.

As can be seen in Fig. 2 the BR signal consists of a very intense and narrow (a few tenths of a ps) peak in the vicinity of zero delay time τ, followed by a much smaller signal of opposite sign that is significantly broader and extends to the delay times up to hundreds of ps. The first intensive peak relates to the temporal overlap of the pump and probe laser pulses. We suggest that this peak was due to the third-order nonlinear Stimulated Raman Scattering (SRS) [7] of the probe beam in water. This signal was observed in our experiments even in pure water without any NADH dissolved. According to our recent theoretical publication [15] the SRS BR effect can be observed only when the pump and probe light frequencies are essentially non-resonant with respect to the excited molecular states that perfectly fits the experimental condition in Fig. 2.

The second, much less intense but significantly broader signal in Fig. 2 can be associated with the linear pump-probe BR effect in NADH. In this case, the pump beam resonantly excited the molecule from the ground state S_g to the first excited electronic state S_1 and the probe beam frequency was out of resonance between the vibrational states v_1 of the first excited electronic state S_1 and the vibrational states v_2 of the second excited electronic state S_2. This signal was not observed in pure aqueous solution and appeared only in the presence of NADH. According to our analysis [15] this signal is characterized by the relatively short vibration relaxation time τ_v and longer rotational diffusion time τ_{rot}.

As can be seen in Fig. 3 the LD signal contains a relatively small signal observed at the delay times τ from a few and up to hundreds of ps. This signal is presented in a smaller scale and longer delay times in the inset. We believe that the wide negative signal in Fig. 3 can be associated with the pump-probe LD effect in NADH. According to the theory [15] and the experimental observation shown in the inset in Fig. 3 this signal has an extremum due to competition of the relatively fast vibration relaxation with the time τ_v and much slower rotational diffusion with the time τ_{rot}.

As can be seen in Fig. 3 the recorded LD signal contains only a relatively small narrow peak in the vicinity of zero delay time τ. We believe that a residual small peak at short delay times in Fig. 3 was due to insufficiently fine alignment of the experimental scheme. The absence of the third-order nonlinear Stimulated Raman Scattering (SRS) signal in Fig. 3 is in perfect agreement with our theoretical prediction [16] that the LD is an essentially resonant effect that could not be observed in pure water in the conditions of our experiments.

Therefore, the experimental data in Figs. 2 and 3 manifest that the POMO-PP method allows for almost complete separation of the contributions from the BR and LD effect that combines with possibility to use relatively small laser pulse energies of a tenths of nJ to obtain high signal-to-noise ratio. As can be seen in Figs. 2 and 3 the method also allows for effective separation of linear pump-probe signals from the third-order nonlinear SRS signals.

5 Conclusion

In this work we developed the POMO-PP method suggested in our previous study for separation of the LD and BR signals and their quantitative determination from experiment. The method was used to acquire LD and BR signals of NADH excited states in aqueous solution. In the case of BR detection both the 3rd order SRS in water and the 1st order pump-probe in NADH were observed. The SRS signal was presented as a very sharp and intensive peak at the delay times of a few tens of ps and the BR pump-probe signal in NADH was presented by a much smaller but wider curve with an extremum. In case of LD detection, the SRS peak in water was practically absent and only the pump-probe signal in NADH was observed. The BR signal in NADH was approximately twice as large than the corresponding LD signal without increasing the pump and probe intensities, thus achieving higher sensitivity for determination of the rotational diffusion times longer than the duration of the SRS peak.

The LD signal was found to be more suitable for the study of the fast vibrational relaxation processes.

Disclosures

The authors declare no conflicts of interest in this paper.

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