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Two-photon absorption spectrum of all-*trans* retinal

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Abstract

The nondegenerate two-photon absorption spectrum of all-*trans* retinal in a nonpolar solvent at room temperature has been measured for the first time. The multichannel detection utilizing the technique of femtosecond time-resolved pump–probe absorption spectroscopy has enabled us to obtain precise two-photon absorption spectra. It was found that the two-photon absorption maximum of all-*trans* retinal is located at 376 nm, which is 7 nm longer than the one-photon absorption peak. This peak wavelength difference indicates the 500-cm^{-1} energy gap between the S_3 (B_u) state and the S_2 (A_g) state. On the basis of the obtained two-photon absorption data and the available fluorescence data, we determined the 0–0 position of the S_2 state to be 2.47×10^4 cm^{-1}.

1. Introduction

The excited electronic structure of the linear polyenes has been attracting considerable interest, and especially the level ordering of the low-lying singlet excited states has been extensively studied [1]. Retinal is one of the most important and interesting linear polyenes, because the isomerization of the retinyl chromophore is the key primary process in several biological functions such as vision. It is known that the isomerization photochemistry of retinal strongly depends on the properties of the excited singlet manifold [2]. In all-*trans* retinal, there are three low-lying excited singlet states (S_1, S_2, S_3) in the energy region up to 30 000 cm^{-1} [3–5]. Femtosecond fluorescence up-conversion spectroscopy [4] has revealed that the S_1, S_2, and S_3 states are of (n, π^*), A_g (π, π^*), and B_u (π, π^*) characters, respectively. The S_3 and S_2 states show ultrafast decay kinetics [4], and they have been identified as the initial states of the all-*trans* → mono-*cis* photoisomerization [5,6]. Although information about the ultrafast dynamics of all-*trans* retinal has been significantly accumulated in these last five years, we still need fundamental spectroscopic data to fully understand the excited-state properties of all-*trans* retinal. For example, even the 0–0 transition energies of the one-photon-forbidden S_2 and S_1 states, which are very important quantity, have not yet been determined.

Two-photon absorption (TPA) spectroscopy is a useful tool to study one-photon-forbidden but

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two-photon-allowed excited states like the A_g states of the polyenes. Obviously, in order to obtain precise information about the A_g states, we need to measure accurate TPA spectra with dense wavelength data points. In conventional measurements of TPA spectra, an intense monochromatic laser beam is focused into a sample, and either TPA or two-photon-excited fluorescence is recorded as a function of the laser frequency. This method is simple and straightforward, but does not readily provide TPA spectra of high quality because of the necessity of scanning the laser frequency. This technical problem in the conventional method is overcome by utilizing white light continuum and a multichannel detector as reported recently by a few groups [7–11].

In this Letter, we apply the multichannel-detection technique of TPA spectroscopy to all-*trans* retinal in hexane. We obtained the TPA spectrum with a very high signal to noise (S/N) ratio, which enabled us to identify and quantify small energy difference between the S_3 (B_u) and S_2 (A_g) states, and to determine the 0–0 transition energy of the S_2 state.

2. Experimental

We first describe the principle of the TPA measurement in the present study. Fig. 1a shows a representative diagram of TPA. We use femtosecond white light visible (VIS) continuum as the ω_2 pulse in order to enable the multichannel detection of TPA spectra. A femtosecond near-infrared (NIR) pulse is used as the ω_1 pulse to induce nondegenerate TPA at $\omega_1 + \omega_2$. We set ω_1 in NIR in order not to bring about degenerate TPA at $2\omega_1$. Because the TPA process occurs only when the ω_1 and ω_2 pulses are temporally overlapped, a TPA spectrum is obtained as an ω_1-induced absorption spectrum at zero time delay between the two pulses. Technically, the present TPA measurement is very similar to femtosecond time-resolved pump–probe absorption spectroscopy. The difference is that we employ a nonresonant NIR pulse for pumping, instead of a resonant UV or VIS pulse. Taking advantage of well-established techniques for femtosecond time-resolved absorption spectroscopy, we can perform the multichannel detection of ω_1-induced transmittance changes in the ω_2 pulse to obtain a TPA spectrum with high S/N. Because of this technical similarity, we call the ω_1 and ω_2 pulses in the present method as the pump and probe pulses, respectively, hereafter. Note that the excited-state population generated in the present nondegenerate TPA measurement is proportional to the product of the pump power and the probe power as indicated by the diagram in Fig. 1a. Therefore, we can hold the excited-state population negligibly small by setting the probe power sufficiently low. This point is an important practical advantage of the present method over the degenerate TPA methods, especially when we study photochemically unstable samples like retinal.

Fig. 1b shows the schematic of the experimental setup for the TPA measurements. A femtosecond Ti:sapphire regenerative amplifier (Spitfire, Spectra Physics) with a femtosecond Ti:sapphire mode-locked oscillator (Tsunami, Spectra Physics) was used as a laser light source. A part of the regenerative amplifier output at 800 nm was focused into water flowing in a 5-mm cell to generate white light continuum, which is used as the probe pulse. An optical parametric amplifier (Topas, Quantronix) was pumped by the other part of the regenerative amplifier output. The signal output of the optical parametric amplifier was employed as the pump pulse. The typical bandwidth of the pump pulse was 300 cm^{-1}. The full width at half maximum of the cross-correlation trace of the pump and probe pulses was about 300 fs. A sample solution in a 1-mm static cell was irradiated by the pump pulse, and the continuum probe pulse passing through the irradiated area of the sample was analyzed by a single polychromator (500im, Chromex) equipped with a thermo-electrically cooled CCD (TEA/CCD-1024-EM/1UV, Princeton Instruments). Wavelength calibration for this multichannel detection system was done by using a mercury lamp and a holmium filter, and the wavelength accuracy was within ± 0.3 nm. The linear polarizations of the pump and probe pulses were set parallel. A pump-induced change in the probe spectrum was recorded as a function of the time delay between the pump and probe pulses.

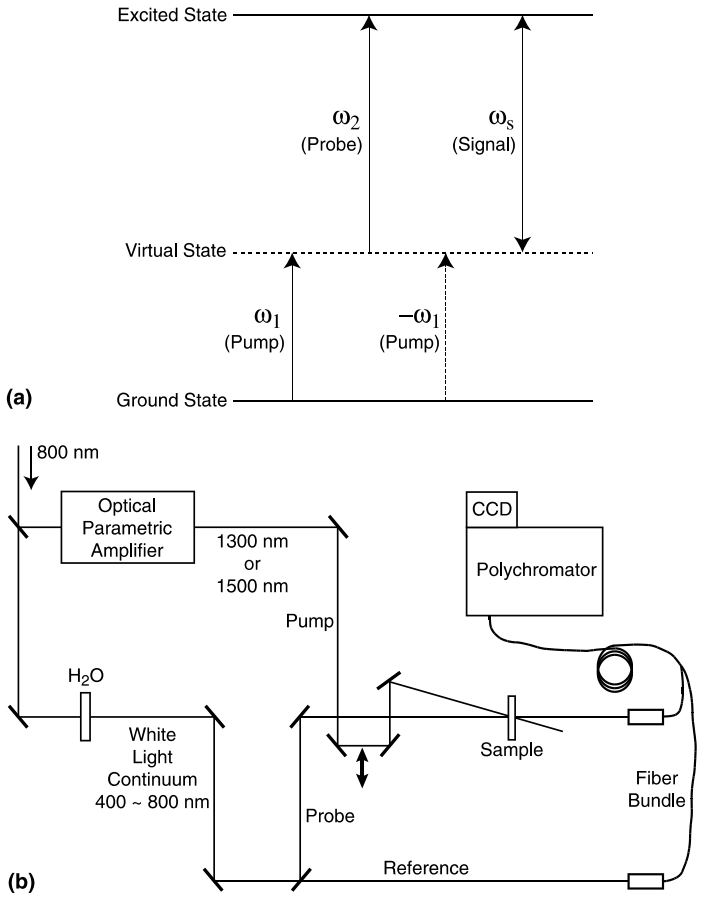

Fig. 1. (a) A diagram of two-photon absorption. The solid and dashed arrows correspond to bra and ket side transitions, respectively. The angular frequency of the signal electric field is expressed as ω_s $(= \omega_1 + \omega_2 - \omega_1)$. (b) Schematic of the two-photon absorption measurement setup.

Although the pump-induced absorption (i.e., TPA) signals were instantaneous, we had to scan the time delay for 2.6 ps because of the frequency chirp in the white light continuum. The effect of the chirp needs to be corrected as in the case of femtosecond transient absorption spectroscopy. The chirp correction was done by using the pump-induced absorption signals themselves [12,13]. The typical measurement time for one TPA spectrum was about 2 h.

All-*trans* retinal (Sigma) and hexane (HPLC grade, Wako) were used as received. The retinal concentration of the sample solution was 3.5×10^{-3} mol dm^{-3}. The sample volume was 5×10^{-4} dm^3. The isomeric purity of the all-*trans* retinal sample was better than 98%. No noticeable increase of the *cis* isomers after the TPA measurements was detected by measuring the *cis*-peak height at 250 nm in the UV absorption spectrum. All the measurements and sample preparations were done in a dark room at room temperature.

3. Results and discussion

Figs. 2a and b show the pump-induced absorption spectra of all-*trans* retinal in hexane at zero time delay. The horizontal axis stands for the probe wavelength. The center wavelength of the pump pulse was 1305 nm for a and 1504 nm for b.

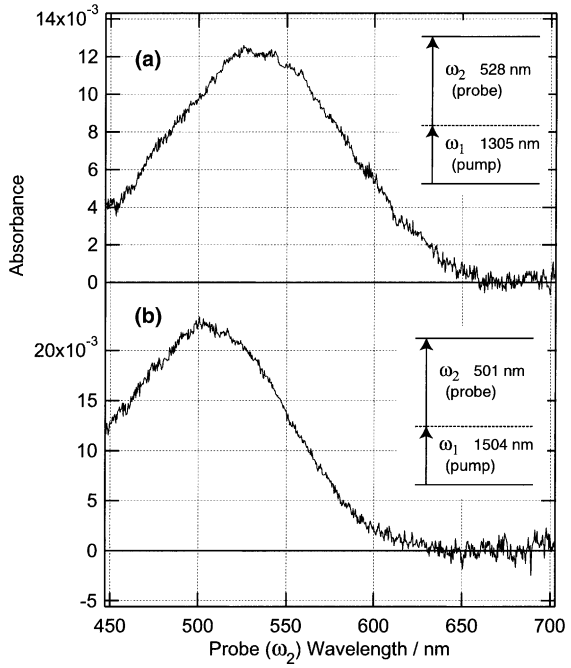

Fig. 2. Pump-induced absorption spectra of all-*trans* retinal in hexane at zero time delay. The pump wavelength is (a) 1305 nm, and (b) 1504 nm. The excitation density of the pump pulse is 27 J m^{-2} for both (a) and (b). The S/N ratios of the absorption spectra have been improved by making the average of the spectra at time delay −100 to +100 fs. The energy diagrams are inserted.

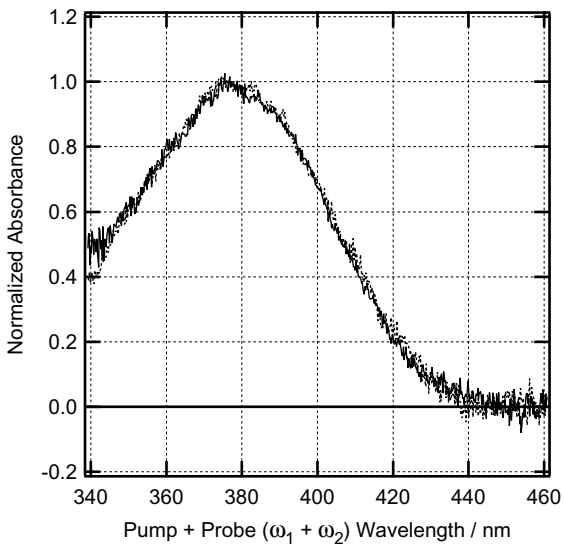

Fig. 3. Pump-induced absorption spectra (the same data as in Fig. 2) plotted as a function of wavelength corresponding to the sum frequency of the pump and probe pulses. The data are normalized. The pump wavelength is 1305 nm for the dotted line, and 1504 nm for the solid line.

The excitation density of the pump pulse was 27 J m^{-2}. Positive signals correspond to an increase of absorbance induced by the pump pulse irradiation. We did not observe any transient absorption signal that was ascribable to the excited singlet or triplet states at any time delay. It means that the pump pulse did not cause degenerate multi-photon excitation. The pump-induced absorption signals appearing in the region of 450–650 nm showed an instantaneous response. In other words, the signals were observed only when the pump and probe pulses had temporal overlap at the sample position. This behavior is consistent with the TPA diagram in Fig. 1a.

The band shape of the spectrum in Fig. 2a is similar to that in b, but the peak wavelengths are different. In Fig. 3, we plot these spectra along the wavelength axis that corresponds to the sum frequency of the pump and probe pulses. (We refer to this axis as 'the $\omega_1 + \omega_2$ wavelength axis', hereafter.) The two spectra obtained with the different pump wavelengths are found to be identical in this plot. This fact represents that the sum frequency of the pump and probe pulses is resonant with an electronic transition of all-*trans* retinal. Therefore, the observed pump-induced absorption signals are ascribed to nondegenerate TPA of all-*trans* retinal.

We measured the pump-power dependence of the absorption signals in order to confirm the above ascription. Fig. 4 shows that the absorbance at probe wavelength of 517 nm is proportional to the excitation density of the pump pulse at 1305 nm. This linear dependence is compatible with the TPA diagram in Fig. 1. It was also confirmed that the band shape of the absorption spectrum was independent of the pump power. Although there exist other third-order nonlinear optical processes such as induced phase modulation, stimulated Raman scattering, and optical Kerr effect, we can expect an unequivocal peak in the UV region (on the $\omega_1 + \omega_2$ wavelength axis) only in the case of TPA. All the results lead us to definitely assign the

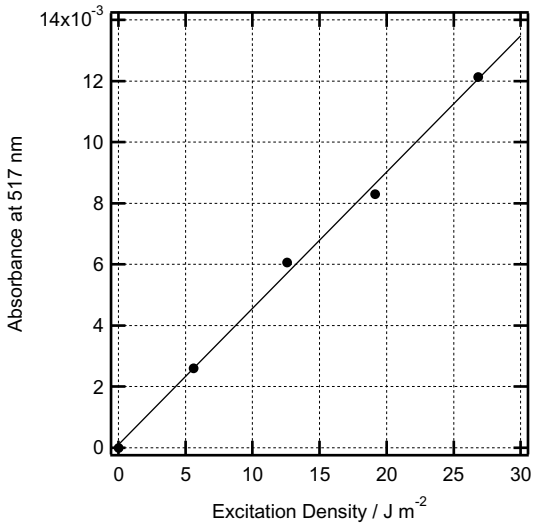

Fig. 4. Excitation density dependence of the pump-induced absorption signal at probe wavelength 517 nm. The pump wavelength is 1305 nm. The solid circles represent data. The line is obtained from fitting.

observed pump-induced absorption signals to TPA of all-*trans* retinal.

The TPA spectrum of all-*trans* retinal is compared with the one-photon absorption spectrum in Fig. 5. As clearly seen, the TPA band is red-shifted compared with the one-photon absorption band. The peak wavelength of the TPA spectrum is determined to be 376 ± 2 nm from Gaussian fitting for the near-peak region, whereas the peak of the one-photon absorption spectrum is at 369 nm. The energy difference between the two peaks is 500 ± 150 cm^{-1}.

As described in Section 1, there are three excited singlet states in the low energy region of all-*trans* retinal. Our femtosecond fluorescence up-conversion study [4] has revealed that the fluorescence of all-*trans* retinal consists of three components, and the peak of the fluorescence spectrum from the S_3 (B_u; π, π^*) state and that from the S_2 (A_g; π, π^*) state are located at $\sim$430 and $\sim$440 nm, respectively. The energy difference between the S_3 and S_2 fluorescence is about 500 cm^{-1}. In addition, the femtosecond fluorescence data indicated that the oscillator strength of the one-photon S_3–S_0 transition is more than 40 times larger than that of

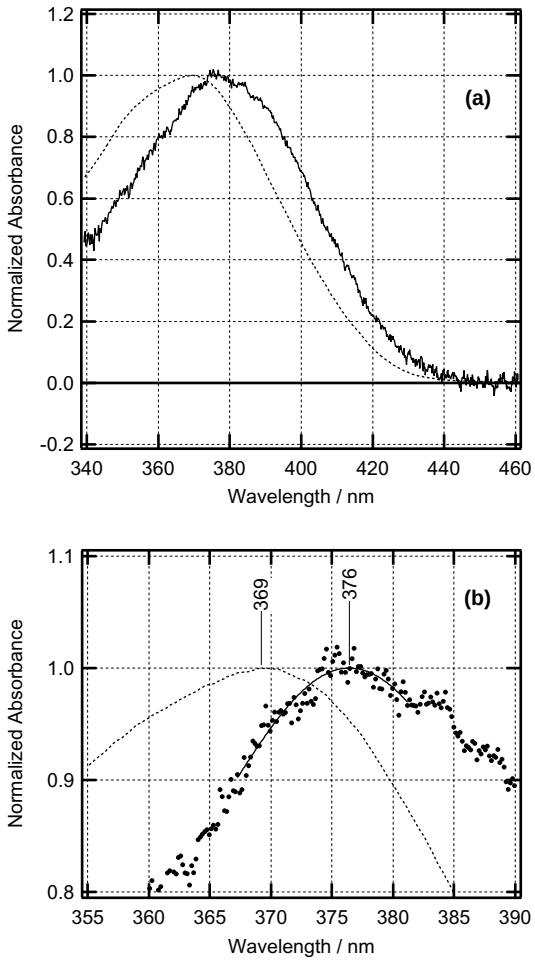

Fig. 5. (a) Comparison of the one-photon (dotted line) and two-photon (solid line) absorption spectra of all-*trans* retinal in hexane. The two-photon absorption spectrum is the average of the two spectra shown in Fig. 3. (b) Peak wavelengths of the one-photon (dotted line) and two-photon (solid circles) absorption spectra. The peak of the latter is determined from Gaussian fitting (solid line) in the near-peak region.

S_2–S_0. It means that the one-photon absorption spectrum is mainly due to the $S_3 \leftarrow S_0$ transition. Although we do not know the relative TPA coefficients of the $S_3 \leftarrow S_0$ and $S_2 \leftarrow S_0$ transitions in a nonpolar solvent, it is natural to consider that the S_2 (A_g) state predominantly contributes to the absorptivity in the TPA spectrum shown in Fig. 5. Actually, the observed energy difference between the one-photon and two-photon absorption peaks

is in very good agreement with that between the S_3 and S_2 fluorescence peaks obtained in the up-conversion study. In this sense, the present TPA data and the femtosecond fluorescence data are consistent, and both of them indicate that the S_3 (B_u) state is located 500 cm^{-1} higher in energy than the S_2 (A_g) state in hexane at room temperature.

The combination of the present TPA data with the femtosecond fluorescence data [4] enables us to determine the 0–0 transition energy of the S_2 (A_g) state, by assuming the mirror-image relation between the absorption and fluorescence spectra. We should note that the mirror-image relation is originally considered to hold between the absorption and the fluorescence from the thermalized electronic excited state. In the case of all-*trans* retinal, however, the lifetimes of the S_3 and S_2 states are so short that the observed fluorescence is from the vibrationally excited S_3 and S_2 states. Nevertheless, this 'hot' fluorescence is considered to appear in almost the same energy region as the fluorescence from the thermally equilibrated S_3 and S_2 states [14], because the highly excited vibrational states in the S_3 and S_2 states have significant Franck–Condon overlap only with highly excited vibrational states in the ground state. Therefore, it is reasonable to assume that the S_3 and S_2 fluorescence spectra observed in the femtosecond up-conversion study hold the mirror images of the one- and two-photon absorption spectra, respectively. The position of the 0–0 transition can be obtained as the energy median of the absorption and fluorescence peaks. The 0–0 transition energy of the S_2 (A_g) state is determined to be 2.47×10^4 cm^{-1} (405 nm), whereas that of the S_3 (B_u) state is 2.52×10^4 cm^{-1} (397 nm). The energy gap is 500 cm^{-1}, which is the same as that evaluated from the peaks of the absorption spectra [1].

[1] For the S_2 state, the absorption peak (376 nm) is located 1900 cm^{-1} higher than the 0–0 transition position (405 nm) in energy. The S_3 state shows the same energy difference between the absorption peak (369 nm) and the 0–0 transition position (397 nm). This means that the vibronic structure of the S_3–S_0 transition is not significantly different from that of the S_2–S_0 transition. Therefore, we can determine the energy gap between the S_3 and S_2 states from the peaks of the one- and two-photon absorption spectra as well as from the 0–0 transition energies.

Birge et al. [3] measured the two-photon fluorescence excitation spectrum of all-*trans* retinal in EPA at 77 K. They reported that the two-photon excitation peak was located at 428 nm, which is significantly longer than the TPA peak observed in the present study. In their work, the energy difference between the one-photon and two-photon absorption was determined to be 2400 cm^{-1}. The discrepancy from the present study is highly likely due to the difference in the experimental condition. Their measurements were performed at low temperature, and EPA is much more polar than hexane and contains ethanol as a hydrogen-bonding donor. Actually, it is known that the molecular structure of all-*trans* retinal depends on temperature [15,16], and that a very small amount of polar or protic solvents such as alcohol or water significantly affects the properties of the excited states of retinal [2,17,18]. We note that the present TPA spectrum is consistent with the results of the resonance hyper-Raman study of all-*trans* retinal [19]. Mizuno et al. measured the excitation profile of the resonance hyper-Raman scattering in hexane at room temperature, but did not observe any enhancement that indicates existence of the A_g state in the energy below 24 400 cm^{-1} (410 nm). This is consistent with the present study, and supports that the S_3 (B_u) and S_2 (A_g) states are very closely located in hexane at room temperature.

On the basis of the spectra in Fig. 2, we can estimate the absolute value of the TPA cross-section of all-*trans* retinal. The peak TPA cross-section for one molecule is estimated at 1.5×10^{-48} cm^4 s $photon^{-1}$ for the 1305-nm pump pulse, and 2.3×10^{-48} cm^4 s $photon^{-1}$ for the 1504-nm pump pulse. Although these two values are deviated, we consider that it is not due to the difference in the pump wavelengths but probably brought about by a wavelength-dependent spatial profile of the pump pulse. The distortion of the spatial profile affects the spatial overlap between the pump and probe pulses, which causes the reduction of the pump-induced absorbance. Therefore, we consider that 1.5×10^{-48} cm^4 s $photon^{-1}$ is the minimum estimation, and regard 2.3×10^{-48} cm^4 s $photon^{-1}$ as a more accurate value. The latter value is $3.3 \sim 7.6$ times higher than the theoretically

calculated TPA cross-section of all-*trans* retinal [3], and it is as high as those of promising optical nonlinear molecules and polymers [7,9]. The large TPA cross-section is consistent with the two-photon-allowed nature of the A_g state.

Finally, we make a comment on the effect of group-velocity mismatch (GVM) in the present TPA measurements. Negres et al. [10,11] pointed out that GVM leads to a temporal walk-off between the pump and probe pulses in the sample, and that the probe-wavelength dependence of the walk-off distorts the TPA spectrum. The effect of GVM becomes larger with an increase in the group-velocity dispersion (GVD) of the sample. Negres et al. showed that adequate correction for GVM was necessary to obtain the TPA spectrum of ZnS that has very large GVD in the VIS region. However, we do not have to take GVM into consideration in the case of transparent organic solutions that have much smaller GVD than ZnS. In the present experiments, the difference between the walk-off time at the probe wavelength of 450 nm and that at 700 nm is estimated to be less than 50 fs. This value is shorter than the pulse width ($=200$ fs), and it is also much shorter than the effective interaction time ($=4.9$ ps) that corresponds to the sample path length. Therefore, the effect of GVM is negligible in the present measurements. In fact, the two TPA peaks observed at the different probe wavelengths in Fig. 2 perfectly coincide on the $\omega_1+\omega_2$ wavelength axis in Fig. 3. It is a clear experimental proof that GVM is not important in the present experimental condition.

In summary, we have performed pump–probe nondegenerate TPA spectroscopy and measured the TPA spectrum of all-*trans* retinal in hexane for the first time. The high S/N ratio and the high density of the wavelength data points have made it possible to evaluate the energy difference between the S_3 (B_u) and S_2 (A_g) states with a high accuracy. The TPA measurements in solvents having different polarity are planned to examine the solvent-polarity dependence of the energy gap between the S_3 and S_2 states.

References

[1] B. Hudson, B. Kohler, Annu. Rev. Phys. Chem. 25 (1974) 437.
[2] R.S. Becker, Photochem. Photobiol. 48 (1988) 369.
[3] R.R. Birge, J.A. Bennett, L.M. Hubbard, H.L. Fang, B.M. Pierce, D.S. Kliger, G.E. Leroi, J. Am. Chem. Soc. 104 (1982) 2519.
[4] S. Takeuchi, T. Tahara, J. Phys. Chem. A 101 (1997) 3052.
[5] S. Yamaguchi, H. Hamaguchi, J. Chem. Phys. 109 (1998) 1397.
[6] T. Yuzawa, H. Hamaguchi, J. Mol. Struct. 352–353 (1995) 489.
[7] N. Pfeffer, P. Raimond, F. Charra, J.M. Nunzi, Chem. Phys. Lett. 201 (1993) 357.
[8] P. Feneyrou, O. Doclot, D. Block, P.L. Baldeck, S. Delysse, J.M. Nunzi, Opt. Lett. 22 (1997) 1132.
[9] G.S. He, T.-C. Lin, P.N. Prasad, R. Kannan, R.A. Vaia, L.-S. Tan, J. Phys. Chem. B 106 (2002) 11081.
[10] R.A. Negres, J.M. Hales, A. Kobyakov, D.J. Hagan, E.W.V. Stryland, Opt. Lett. 27 (2002) 270.
[11] R.A. Negres, J.M. Hales, A. Kobyakov, D.J. Hagan, E.W.V. Stryland, IEEE J. Quantum Electron. 38 (2002) 1205.
[12] R.L. Fork, C.V. Shank, C. Hirlimann, R. Yen, W.J. Tomlinson, Opt. Lett. 8 (1983) 1.
[13] S. Yamaguchi, H. Hamaguchi, Appl. Spectrosc. 49 (1995) 1513.
[14] N. Sarkar, S. Takeuchi, T. Tahara, J. Phys. Chem. A 103 (1999) 4808.
[15] G. Drikos, P. Morys, H. Rüppel, Photochem. Photobiol. 40 (1984) 133.
[16] S.A. Locknar, A. Chowdhury, L.A. Peteanu, J. Phys. Chem. B 104 (2000) 5816.
[17] T. Takemura, P.K. Das, G. Hug, R.S. Becker, J. Am. Chem. Soc. 100 (1978) 2626.
[18] S. Yamaguchi, H. Hamaguchi, J. Phys. Chem. A 104 (2000) 4272.
[19] M. Mizuno, H. Hamaguchi, T. Tahara, J. Phys. Chem. A 106 (2002) 3599.