Photothermal Lensing Signal Enhancement by the Transient Absorption of Photoexcited States in Liquid Solutions

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A two-color double-pulse excited photothermal lensing measurement was performed to investigate a signal enhancement owing to transient absorption by the excited states of solute molecules in liquid solutions. A colinear, mode-mismatched configuration with a continuous-wave probe laser at 633 nm was adapted with a focused pump pulse (6.9 ns, 355 nm) and a sequentially incident softly focused second-pump pulse (7.2 ns, 532 nm). The signal-intensity dependence on the energies of the pump and the second-pump pulses was measured for 1.0 μM solutions of 2,2'-*p*-phenylenebis(5-phenyloxazole) in 1-butanol and anthracene in ethanol. A signal enhancement was observed for both solutions. The signal intensity under certain conditions was one order higher than that expected from ground-state absorption. A discussion was made on determination of the absorption coefficient of the transient species and on the absorption saturation of the ground and excited states.

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Introduction

A photothermal lensing (PTL) measurement is one of the most sensitive methods for detecting chemical species, especially non-luminescent substances, in both gas and condensed phases. This method has been used as a suitable investigating tool in a wide range of application fields.¹ For photothermal methods like photoacoustic spectroscopy, PTL spectroscopy, photothermal deflection spectroscopy, and others, the signal magnitude is directly related to the temperature rise in a light-illuminated material and to the amount of heat generated *via* optical absorption and subsequent nonradiative relaxation. One of the main factors resulting in good sensitivity is that stronger excitation light causes a larger heat-generation rate. Another factor is that heat accumulation for longer time results in a larger temperature rise. In addition, PTL methods are effective for small volumes of heat accumulation, because the spatial gradient of the refractive index, which is coupled to the temperature gradient by the thermo-optic-coupling constant, influences the sensitivity. Crossed-beam and colinear configurations have been applied to probe small volumes. The detection limit of target molecules in liquid solution at room temperature has been improved to the level of one hundred molecules in 200 fL² and in 1.3 fL³ and less than one molecule in 3 fL⁴ of the probe volume. However, molecules in a liquid move around so fast that these values of the molecule numbers are time-averaged. Single-molecule detection in liquid solutions, where molecules are counted one by one, has been realized by means of fluorescence measurements.^{5,6} As for nonfluorescent substances in a liquid, the counting of single colloidal silver particles with a diameter of 10 nm has been carried out by PTL measurements under a microscope.⁷ For nonfluorescent small

molecules in solution, electrochemical methods can detect single molecules.⁸ Surface-enhanced Raman scattering may be applied if the adsorbed molecule on a silver nanoparticle is the target molecule.⁹ However, some technical improvements are still required to count small nonfluorescent molecules in solution.

Generally, the PTL signal of a dilute solution linearly increases with the excitation light intensity, but under a focused laser beam, the saturation of optical absorption easily takes place and limits the PTL-signal increase, even if the excitation intensity increases. The absorption saturation, settling the upper limit of the photo-induced heat generation rate per single target molecule, limits the sensitivity of detection. To obtain a large PTL signal beyond the limitation, the use of transient absorption (optical absorption by molecules in photoexcited states) can be the best method. Heat generation *via* transient absorption has been observed and applied to investigate the dynamics of molecules in dilute solution, where sequential pulsed excitation was used in combination with photothermal detection.¹⁰⁻¹³ For transient absorption, high efficiency is expected of photon energy conversion into heat, because highly excited molecules in liquid rapidly and nonradiatively relax to the lowest vibrational level of the lowest electronic excited state. Moreover, multiple roundtrips in molecular states between the lowest electronic excited level and a higher level may occur during light illumination for the target molecules in a liquid solution. For molecular solids, the roundtrips are known to be one of the important mechanisms of optical ablation under high-intensity laser illumination, where extremely large energy is optically deposited in the material at a time.¹⁴

In this work, we studied PTL-signal enhancement using transient absorption, by the two-color sequential double-pulsed excitation of solute molecules in liquids. It was found that the signal intensity under certain conditions was one order higher than that expected from the ground-state absorption. First, a theoretical explanation for the PTL-signal enhancement is described, while discussing a mode-mismatched colinear-type

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configuration of three laser beams focused on the sample to relate the transient absorption coefficient to the magnitude of the signal enhancement. The experimental details and results are given next.

Theory

Saturation of PTL signal by optical absorption saturation

Under intense laser irradiation, the absorption coefficient (α) is known to depend on the light intensity (I) as $\alpha(I) = \alpha_0/(1 + I/I_s)$, where α_0 is the unsaturated absorption coefficient. The absorption saturation threshold (I_s) indexes when the saturation becomes serious, and is given by $I_s = h\nu/2\sigma\tau_{\text{eff}}$, where $h\nu$ is the photon energy, σ is the absorption cross-section of the molecule, and τ_{eff} denotes the average value of the photoexcited state lifetime during the irradiation.¹⁵ The absorption saturation is significant for focused pulsed-laser beam irradiation (duration t_p and radius w), even if the energy per pulse (E) is small, because $I = E/\pi w^2 t_p$ is large.

In a material under focused pulsed-laser irradiation, a part of the light energy is converted into heat, causing the temperature to jump at the laser spot. A refractive-index gradient, which mimics the temperature gradient, is formed around the spot and works as a lens element. The lens-like element (thermal lens) is detectable with a probe beam passing through the spot by monitoring the change in the divergence.¹ With a well-arranged experimental setup, the PTL-signal intensity (Q) has a linear relation with the amount of photo-generated heat, which is proportional to αE under a small-absorption condition, *i.e.*, $\alpha \ll 1 \text{ cm}^{-1}$. The above relations provide the following equation for PTL-signal saturation:

$$Q = Q_\infty E/(E + E_s), \quad (1)$$

where $E_s (= \pi w^2 t_p I_s)$ is the pulse energy at the saturation threshold. It is obvious that Q cannot exceed a finite value (Q_∞) even at large E . Namely, the PTL signal suffers saturation under high-intensity excitation.

PTL signal for a mode-mismatched co-linear configuration of three laser beams

There are a variety of experimental setups for PTL-signal measurements.^{1,16} PTL-signal enhancement is commonly expected whenever transient absorption is utilized. However, for convenience, we present below a certain PTL setup of a mode-mismatched co-linear configuration. We consider three laser beams of different wavelengths (ultraviolet, green, and red), different diameters (large, small, and large) and different types (pulse, pulse, and continuous wave). These beams are identified as pump, second-pump, and probe beams, respectively. When all of the beams propagate along the same axis (z -axis) and are focused by a convex lens into a sample cell, the beam paths near their focal points are schematically sketched in Fig. 1, where thick, dotted, and thin curves represent the pump, second-pump, and probe beams, respectively. The beam propagation is perpendicular to the x - y plane, and the origin of the system (o) is placed at the focus of the probe (red) beam. The PTL effect induced by the pump beam is monitored with the probe beam. Transient absorption by the second-pump beam, incident after the pump beam, causes the PTL-signal enhancement.

A chromatic aberration of the convex lens makes the three beams mode-mismatched. In other words, the positions of their focal points are different, and they generally have different beam sizes at the focal points. The configuration shown in Fig.

Fig. 1 Schematic illustration showing the overlap of three beams with different wavelengths, convergences, and focus positions, near their focuses.

1 results from the fact that larger diameter beams at the front of the convex lens are more tightly focused and shorter wavelength beams have focal points closer to the lens and smaller diameters. The point of this beam configuration is such that the second-pump beam has a sufficiently small divergence and a sufficiently large radius to provide nearly homogenous illumination around the focal point of the pump beam. The spatial distribution of the heat source by the pump beam has the same shape as that caused by the transient absorption of the second-pump beam, because the distribution of the photoexcited species has the same shape as the heat source.

When a small-area photodiode is used to measure the probe-beam intensity at a position on the axis ($x = y = 0$) and at a large z , the PTL effect is observed as an increase in the photocurrent intensity. This is understandable for a thin sample, in which the intensity increase is proportional to $-z_0(dn/dT)(\partial^2 T/\partial r^2)_{r=0}$, where $z_0 (<0)$ is the position of the sample, n is the refractive index, T is the temperature, and r is the distance from the z -axis.¹ Even for the thick sample considered for the configuration above, we can conclude that most of the PTL effect is caused in the region near to the focal point of the pump beam, where photo-induced $(\partial^2 T/\partial r^2)_{r=0}$ is the largest and z_0 has a non-zero value. Although the second-pump beam, itself, may generate heat to cause the PTL effect by ground-state absorption, the spatial distribution of the heat is different from that by the pump beam. A relatively small contribution is expected because the size of the beam is large.

Signal enhancement by transient absorption

Here, we consider the PTL signal under independent or sequential irradiation to the sample by the pump and second-pump pulses. The independent irradiation of the pump (second-pump) light generates a PTL signal of magnitude Q_1 (Q_2) by ground-state absorption. If the second-pump beam irradiates after the pump beam, the magnitude of the PTL signal (Q) would be

$$Q = Q_1 + Q_2 + Q_{12}, \quad (2)$$

where Q_{12} represents the contribution of the transient absorption to the signal. The gain of the PTL signal by the second-pump beam is important for the practical purpose of sensitive detection with species selectivity. We define the gain as

$$\text{Gain} = Q/(Q_1 + Q_2). \quad (3)$$

The gain value is theoretically available in a simple way when the absorption saturation effect is neglected. Consider a sample solution with a solute concentration of C (M) and a molar absorption coefficient of the solute ϵ_i ($\text{cm}^{-1} \text{ M}^{-1}$). Subscript i of 1 or 2 is used to represent the pump or the second-pump beam, respectively. The sample is irradiated by light pulses with pulse

energy E_i (mJ/pulse), beam radius w_i (cm²), and temporal shapes $g_i(t)$ (s⁻¹) with $\int_{-\infty}^{\infty} g_i(t)dt=1$. When the efficiency (η_i) of the light energy converting into thermal energy is considered, the magnitude of PTL signal by single-beam irradiation is expressed as

$$Q_i = GA_i\eta_i E_i w_i^{-2} \varepsilon_i C, \quad (4)$$

where G is the gain factor of the electronics. A_i (cm³/mJ) is a proportionality constant which depends on the thermo-optic-coupling constant (dn/dT) and the geometrical configuration between the i -beam, the probe beam, and the sample. Generally, A_1 is unequal to A_2 because of the different geometry.

When the second-pump beam irradiates the sample just after pulsed illumination of the pump beam, the time-dependent concentration of the photoexcited molecule $C_{ex}(t)$ (M) should be considered, and we obtain

$$Q_{12} = GA_1\eta_{12}E_2w_2^{-2}\varepsilon_{12}\int_{-\infty}^{\infty} C_{ex}(t)g_2(t)dt, \quad (5)$$

where ε_{12} (cm⁻¹ M⁻¹) is the molar-absorption coefficient for the transient absorption and η_{12} is the efficiency of light energy converting into thermal energy for the relaxation process from a highly photoexcited state to the lowest electronic photoexcited state. We can use the proportionality constant (A_1) in Eq. (5) because of the reason discussed before. Although the concentration of the photoexcited molecules is a function of space, no generality is lost when we use values on the beam axis and at the pump-beam waist to calculate the concentration. At this point, the light intensity of the pump pulse is given by $2E_1g_1(t)/w_1^2$ (mW/cm²), and if the excited state has a single lifetime of τ , then

$$C_{ex}(t) = B_1E_1w_1^{-2}\varepsilon_1C\int_{-\infty}^t g_1(t')e^{-(t-t')/\tau}dt', \quad (6)$$

where $B_1 = 2 \times 10^3 \ln(10)/(\pi N_A h \nu_1)$ (M mJ⁻¹ cm³), $h\nu_1$ (mJ) is the photon energy of the pump pulse, and N_A is Avogadro's number. We can use Eqs. (2) – (6) to obtain the relations

$$\frac{Q-Q_2}{Q_1} = 1 + \chi \frac{\eta_{12}\varepsilon_{12}}{\eta_1} E_2, \quad (7)$$

and

$$Gain = 1 + \frac{1}{1+Q_2/Q_1} \chi \frac{\eta_{12}\varepsilon_{12}}{\eta_1} E_2, \quad (8)$$

where χ (M cm mJ⁻¹) is a proportionality constant given by

$$\chi = B_1w_2^{-2}\int_{-\infty}^{\infty} dt \int_{-\infty}^t dt' \times g_2(t)g_1(t')e^{-(t-t')/\tau}. \quad (9)$$

The value of χ can be calculated for a given experimental condition. η_1 is a known value for the ground state that is estimable from fluorescence quantum efficiency and the Stokes shift in the emission spectrum.¹⁷ Generally, we can assume $\eta_{12} = 1$. Thus, Eq. (7) demonstrates that ε_{12} can be experimentally determined. Equation (8) indicates that a larger gain can be obtained with stronger E_2 because Q_2/Q_1 is negligible for most cases of interests to us.

It should be noted that the absorption saturation effect is not taken into account in Eqs. (4) – (9). Some modification is

Fig. 2 Schematic illustration of the experimental setup for thermal lensing signal enhancement using transient absorption. A, ails; BD, beam dump; DM, dichroic mirror; F, colored glass filter; GP, glass plate; ND, neutral density filter; PD, photodiode; PH, pinhole; and WP, half-wave plate. A set of WP and GP was used for adjusting pump-beam intensity.

required under the strong pump- or the strong second-pump condition. If the saturation of transient absorption becomes serious at a strong E_2 , the ($Gain-1$) behaves as the E -dependence in Eq. (1). Although the saturation of ground-state absorption can interfere with the gain, it is not very simple to obtain a useful analytical expression of the saturation effect (see Appendix). It is obvious from Eq. (8) that the gain is independent of E_1 when Q_2/Q_1 is negligible, making the influence of the ground-state absorption saturation to the gain appear in higher order terms.

Experimental

A schematic illustration of the experimental setup for PTL-signal enhancement using transient absorption is shown in Fig. 2. The third (355 nm) and second (532 nm) harmonics of a Q-switched Nd-doped yttrium aluminum garnet laser (Surelite I-10, Continuum) was used as pump and second-pump beams, respectively. The pulsed laser was operated at 10 Hz of repetition frequency. The intensity of the pump beam was adjusted with a half-wave plate and Brewster's angle reflector of the glass plate, and that of the second-pump beam was adjusted with a set of neutral-density filters. The polarization planes of the beams were set parallel to each other. A continuous-wave He-Ne laser (632.8 nm, Model 1101P, Uniphase) was used as a probe beam combined with a spatial filter made up of an objective lens ($\times 10$, NA 0.25), a 25- μ m pinhole, and a convex lens. These three beams were colinearly combined with two dichroic mirrors after they passed through irises of appropriate sizes. They were focused into a quartz sample cuvette by an achromatic convex lens with a focusing length of 200 mm (UDL-30-200P, Sigma-Koki). The beam parameters are listed in Table 1, and a detailed discussion is provided later. The probe-beam intensity on the optical axis was monitored at 500 mm from the sample cell, with a photodiode connected to an isolation amplifier (P-62, NF circuit design block Co.). In front of the photodiode, a red-colored glass filter (SCF-50S-62R, Sigma-Koki) and an interference filter (632.8 ± 5.5 nm, Edmund Scientific) were placed to cut off the pump and second-pump beams. The output showed a transient signal increase and succeeding decay in 20 ms when the pump pulse illuminated the sample. A box-car integrator (BX-530A, NF circuit design block Co.) was used to hold the peak intensity. The accumulated box-car output was recorded as the PTL-signal intensity. The temporal shapes of the optical

Table 1 The laser-beam parameters of the present experiment using the focusing lens (UDL-30-200P, Sigma-Koki)

	Wavelength/ nm	Focal length ^a / mm	Diameter/ μ m			Confocal length ^b /mm	Pulse width ^c / ns
			at entrance apertures	at beam waists ^b	at the pump beam waist ^b		
Pump	355	204.9	6500	14.2	14.2	0.45	6.9
2nd-pump	532	206.6	2300	60.8	63.9	5.47	7.2
Probe	632.8	206.9	6000	27.8	64.3	0.96	(CW)

a. Calculated with reference data in Ref. 18. b. Calculated by assuming Gaussian laser beams. c. Measured values of full-width at the half-maximum.

Fig. 3 Calculated intensity distribution of the three laser beams (a) in the focal plane of the 355-nm pump beam as a function of distance from the optical axis; and (b) on and along the optical axis.

pulses were measured with a fast PIN photodiode and a 500-MHz digitizing oscilloscope (TDS-520C, Tektronix).

The samples used were a 1-butanol solution of 2,2'-p-phenylenebis(5-phenyloxazole) (POPOP) and an ethanol solution of anthracene. The typical concentration was 1.0 μ M. The absorption coefficient at 355 nm was 0.02 cm^{-1} and that at 532 nm was negligibly small for both 1.0 μ M POPOP and 2.5 μ M anthracene solutions. Anthracene, ethanol, and 1-butanol of special grade and POPOP for scintillation were purchased from Kishida Chemical Co., Ltd. and used without further purification.

Results and Discussion

Characteristics of the laser beams in the PTL measurement

Because the PTL signal in the two- and three-color experiments strongly depends on the spatial overlaps of the laser beams, it is important to specify the beam parameters (position of focus and beam diameters at certain positions) for each laser beam before discussing any experimental results. Because the well-characterized convex lens of UDL-30-200P was used to focus the laser beams with small divergence, the positions of the focuses were estimated with values in the characteristic data table¹⁸ of the wavelength-dependent focal length of the lens. We calculated the beam radius at certain positions (Table 1) and intensity distributions (Fig. 3). We assumed that all beams had Gaussian intensity distributions in their cross-sections, and that their diameters at the lens were the

Fig. 4 (a) Measured transient waveforms of the pulsed-laser beams: accumulation was for 512 pulses; delay time of the visible light was 4.8 ns with respect to the ultraviolet light. (b) Transient waveforms calculated as Gaussian temporal profiles separated by 4.8 ns. The population transient of the S_1 -state with 5.0 ns of lifetime excited by the ultraviolet light pulse is also shown.

same sizes as those of the entrance apertures. Because $(\partial^2 T / \partial r^2)_{r=0}$ is important for the PTL effect, most of the effect is generated in the region where the pump-beam intensity is large, namely 204 – 206 mm from the focusing lens. We confirmed that the 532-nm second-pump beam would have nearly homogeneous intensity in the paraxial region around the focal point of the 355-nm pump beam, as shown in Figs. 3 (a) and (b).

Figure 4(a) shows the temporal shapes of pulsed beams averaged over 512 pulses for both the 355-nm pump pulse and the 532-nm second-pump pulse. Although both pulses had fluctuating multiple peaks separated by a 2-ns time interval when we observed the shapes of single pulses, the averaged shapes were in a Gaussian-like distribution with weak tails. The temporal shapes were well expressed as

$$g_1(t) = \sqrt{\frac{2}{\pi}} \frac{1}{\tau_1} \exp[-2(t/\tau_1)^2] \quad (10)$$

and

$$g_2(t) = \sqrt{\frac{2}{\pi}} \frac{1}{\tau_2} \exp[-2\{(t-t_d)/\tau_2\}^2], \quad (11)$$

Fig. 5 Pump-pulse energy dependence of thermal lensing signal intensity for 1.0 μM solutions of POPOP in 1-butanol and anthracene in ethanol. The solid curves are fitting results showing saturation thresholds of 0.068 mJ/pulse and 0.15 mJ/pulse for POPOP and anthracene, respectively.

where t_d is the delay time of the second pulse with respect to the pump pulse, and $\tau_1\sqrt{2 \ln 2}$ ($\tau_2\sqrt{2 \ln 2}$) is the full width at the half-maximum of the pump (second-pump) pulse. The pulse widths were calculated by a fitting procedure, and are listed in Table 1. The value of t_d was 4.8 ns. In Fig. 4(b), the normalized shapes of the Gaussian beams are shown together with the calculated population transient of the lowest photoexcited singlet state S_1 with a lifetime of 5.0 ns as an example. The proportionality constant (χ) is dominated by the overlap between the second-pump pulse and the S_1 population. It is apparent that by changing the timing delay, we can optimize the large gain, although we did not do so in the present experiment. The proportionality constant (χ) was calculated with reference values having lifetimes of 1.3 ns for POPOP in ethanol¹⁹ and 5.0 ns for anthracene in ethanol^{19,20} to be 3.6×10^{-2} and 1.4×10^{-1} (M cm mJ^{-1}), respectively. These values are used in a later discussion.

Saturation of the PTL signal by the pump pulse

The pump-pulse energy dependence of the PTL-signal intensity is shown in Fig. 5 for both the 1.0 μM POPOP and 1.0 μM anthracene solutions. The saturation of optical absorption can easily result from the pulsed excitation of a strong absorption band, and the dependence tends to deviate from a linear relation. The deviation was attributed to absorption saturation because the absolute signal intensity was small enough to neglect the intrinsic nonlinear behavior of the PTL signal (the change in the probe light intensity detected by the photodiode was less than 2.4% of the unperturbed intensity, even at the maximum PTL-signal intensity). The solid curves in Fig. 5 provide the best fitting to Eq. (1). The saturation threshold (E_s) was determined to be 0.068 mJ for POPOP and 0.15 mJ for anthracene. These values corresponded to 0.59 ps and 0.68 ps of τ_{eff} , respectively, where the σ used were $7.7 \times 10^{-17} \text{ cm}^2$ for POPOP and $3.1 \times 10^{-17} \text{ cm}^2$ for anthracene. Although these τ_{eff} values were much smaller than the fluorescence lifetimes of either compound, they are not surprising, because τ_{eff} -values of the same order have been reported for a dye in poly(methyl methacrylate) by near-infrared absorption.¹⁵ Any inaccuracies in the beam size and pulse width could have some influence on the quantitative evaluation of τ_{eff} . The spatial distribution of the beam intensity and the temporal shapes of the light pulse could cause an underestimation of τ_{eff} . Besides these, it is not easy to provide a theoretical explanation for a discrepancy of three orders of magnitude, even when applying a complex model of dynamics that takes the

Fig. 6 Enhancement of thermal lensing signal by sequential incidence of pump and second-pump pulses. Transient waveforms pumped by ultraviolet pulse only, by visible pulse only and by both ultraviolet and visible pulses are shown. The sample was 1.0 μM POPOP solution in 1-butanol.

Fig. 7 Second-pump pulse energy dependence of thermal lensing signal intensity for 1.0 μM POPOP solution in 1-butanol. The solid curve for the 0.03-mJ/pulse pump condition is a fitting result showing an absorption saturation threshold of 0.31 mJ/pulse. The solid line for the no-pump condition is the result of linear regression.

degeneracy of the ground and excited states into account. However, it is sufficient here to note that the PTL signal showed saturation when the pump laser intensity increased, and that signal enhancement by transient absorption is one possible way to overcome the saturation limit.

Enhancement of the PTL signal by the second-pump pulse

Signal enhancement by the second-pump pulse is clearly demonstrated in Fig. 6. Typical traces of PTL-signal transients are shown for the 1.0 μM POPOP solution. The POPOP dye has strong optical absorption at 355 nm and weak absorption at 532 nm. A larger enhancement was achieved with an intense 532-nm pulse following a weak 355-nm pulse, while independent irradiation of the 532-nm pulse or the 355-nm pulse generated small signals. The PTL-signal intensity depended on the second-pump pulse energy, as shown in Fig. 7, while the pump-pulse energy was fixed to 0.03 mJ/pulse. The signal obtained with no pump pulse was due to weak absorption at 532 nm. The signal intensity at zero of the second-pump pulse energy was caused by the pump beam at 355 nm. If there were no transient absorption at 532 nm, a simple summation of signal intensities should be obtained with the two-color excitation. A much larger signal observed was attributed to transient absorption by photoexcited molecules.

The transient absorption of POPOP also showed a saturation behavior, as in Fig. 7. The solid curve in Fig. 7 shows the best fitting to Eq. (1), with which we estimated the saturation

Fig. 8 Second-pump pulse energy dependence of gain in the thermal lensing signal intensity for 1.0 μM solutions of POPOP in 1-butanol and anthracene in ethanol. Pump-pulse energy was 0.03 mJ/pulse for POPOP and 0.007 mJ/pulse for anthracene, respectively.

Fig. 9 Pump-pulse energy dependence of gain in the thermal lensing signal intensity for 1.0 μM solutions of POPOP in 1-butanol and anthracene in ethanol. Second-pump pulse energy was 0.60 mJ/pulse for POPOP and 0.62 mJ/pulse for anthracene, respectively.

threshold of transient absorption to be 0.31 mJ. With the molar absorption coefficient, $\epsilon_{12} = 7.5 \times 10^3 \text{ (cm}^{-1} \text{ M}^{-1})$ ²¹ for a transient absorption of POPOP at 532 nm, we estimated $\tau_{\text{eff}} = 4.7 \text{ ps}$. This value was 8 times larger than that of ground-state absorption saturation. POPOP dyes are excited to the S_1 states by 355-nm light²² and the molecules in S_1 states are excited to the S_3 states by 532-nm light.²² Although the lifetime of the highly excited state is unknown for POPOP, it is generally expected to be less than several picoseconds for such dye molecules in a liquid solution at room temperature. We concluded that there is no simple relation between the τ_{eff} values and the lifetimes of the ground and excited states, and that it is necessary to evaluate the saturation threshold experimentally, although the saturation threshold is one of the decisive factors to achieve a higher gain.

Figure 8 shows the gain dependence on the second-pump pulse energy. Note that first, the gain was over 10 for POPOP under an appropriate condition, and second, the gain depended on the kind of molecular species. These results are suggestive when ultra-sensitive and species-selective detection is conducted for the ultratrace analysis of a sample in a condensed phase. A larger gain is expected as the second-pump pulse energy increases. Besides absorption saturation, the gain will be limited by some nonlinear optical phenomena, such as the optical breakdown of a solution (or cell wall) or stimulated Raman effects of the solvent, but the experimental conditions can be optimized to achieve high sensitivity. The data plots in Fig. 8 for anthracene pumped with 0.007 mJ at 355 nm are on a straight line up to 0.62 mJ of second-pump pulse energy. Since Q_2/Q_1 was negligible for anthracene, the linear dependence is that expected from Eq. (8). The molecular properties of anthracene and the experimental conditions would cause good linearity based on the rapid depletion of molecules in the excited singlet state and have no influence on the triplet- and ground-state absorption at the second-pump wavelength. Anthracene is a molecule with a high triplet-formation quantum yield of 0.7 in ethanol.²³ The forbidden transition from the singlet ground state to the lowest excited triplet state around 550 nm has an absorption coefficient smaller than $10^{-4} \text{ (cm}^{-1} \text{ M}^{-1})$.²⁴ Small transient absorption is expected at 532 nm by triplet-triplet transitions that lie above 520 nm of photon energy in polymethyl-methacrylate²⁵ and around 430 nm and above 460 nm of photon energy in cyclohexane and benzene.²⁶ The lifetime of the triplet state is much shorter than 100 ms of the repetition rate. For POPOP, a linear dependence of the gain

was observed only for small values of the second-pump pulse energy, and the gain was saturated for large energy. The slope of the initial linear portion was $46 \text{ (mJ}^{-1})$. The slope of the line for anthracene was $7.3 \text{ (mJ}^{-1})$.

We estimated η_1 of 0.20 and 0.81 for POPOP and anthracene, respectively.²⁷ These values correspond to $\chi\eta_{12}/\eta_1$ of $1.8 \times 10^{-1} \text{ (M cm mJ}^{-1})$ for both POPOP and anthracene. From the slope of the gain curve, ϵ_{12} was estimated to be $2.6 \times 10^5 \text{ (cm}^{-1} \text{ M}^{-1})$ for POPOP and $4.1 \times 10^4 \text{ (cm}^{-1} \text{ M}^{-1})$ for anthracene. The experimental value of ϵ_{12} for POPOP was 35-times larger than the reference value, $7.5 \times 10^3 \text{ (cm}^{-1} \text{ M}^{-1})$, at 532 nm. Unfortunately, we could find no direct reference of ϵ_{12} for anthracene. The ϵ_{12} value was estimated to be $2 \times 10^3 \text{ (cm}^{-1} \text{ M}^{-1})$ at 532 nm, the same value as for 9,10-diphenyl-anthracene, because the shapes of the transient absorption spectra between 500 and 600 nm were similar for both molecules with the same chromophore.^{21,28} The value $\epsilon_{12} = 4.1 \times 10^4 \text{ (cm}^{-1} \text{ M}^{-1})$, which was determined experimentally, is significantly large. It is possible to underestimate χ by a possible inaccuracy in ω_2 , although, to compensate the 35-times discrepancy, we need the implausible assumption of a 5.9-times larger ω_2 . A large η_1 can somewhat lessen the discrepancy, but it seems that multiple-time photoexcitation of certain molecules in the photoexcited states took place to enlarge the effective ϵ_{12} .

Figure 9 shows the pump-pulse energy dependence of the gain, where the second-pump pulse energy was fixed to 0.6 mJ. The gain increased as the pump intensity decreased. It gradually decreased with increasing pump-pulse energy, and at high pump conditions the gain was 5.4 and 1.5 for POPOP and anthracene, respectively. The pump-intensity-dependent behavior of the gain curves is beyond the model described before. It might be considered that the behavior is caused by the pump-pulse energy-dependence of χ , because under strong pump conditions the time-evolution of the photoexcited species concentration would be different from that under weak pump conditions. Another possible reason could be found in η_1 under the strong pump condition. However, no conclusive reasons for the mechanisms were found, even with a modified model given in Appendix, and further investigation is required. It is suggestive that the pump-pulse energy above which the gain achieved a constant value corresponded to the saturation threshold of the ground-state absorption for POPOP. This seems reasonable in view of the fact that, above the saturation threshold, the population of excited-state molecules does not change appreciably. However, for anthracene, whose yield of

intersystem crossing is 0.7,²³ a simple explanation, such as that for POPOP above, is inappropriate because the triplet-state formation causes a rapid depletion of the ground-states population.

It is true that mechanisms with higher complexity than our simple theoretical model may explain the PTL-signal enhancement better, but our model is a good guide for designing a sensitive and selective detection system. A preliminary experiment of the PTL measurements in a capillary cell with 0.2-mm optical path length successfully showed signal enhancement of the same order of magnitude and of the same species-dependency. We think that the use of continuous-wave lasers for the pump and second-pump beams is preferable for PTL signal enhancement because optical damage of the cell wall easily takes place for the pulsed laser beams, especially for the capillary cell. Even when focused continuous-wave laser beams are used for excitation, the absorption saturation can be serious for the PTL signal, and our model is applicable with some modification.

Conclusion

A two-color sequential double-pulse excited PTL measurement was performed to investigate any signal enhancement owing to transient absorption by excited solute-molecules in liquid solutions. A colinear, mode-mismatched configuration was adapted with a focused ultraviolet pump pulse and a sequentially incident softly focused visible pulse designed so as to allow the determination of the effective absorption coefficient of the transient species. A more than one order large signal was obtained in a 1.0 μM POPOP solution in hexane, and a species-selective enhancement was demonstrated. Effects of the absorption saturation for the ground state and the excited state were discussed. The method demonstrated here promises to be crucial for counting non-fluorescent single molecules in liquid solutions and for finding the upper limit in accumulating the photo-induced heat energy during a certain time interval and in a certain space of controlled dimension.

Appendix

As a modified model explaining PTL-signal saturation caused by optical absorption saturation, we can use a two-level system assuming degeneracy in each level. By considering a constant total concentration of $C = C_{\text{gr}}(t) + C_{\text{ex}}(t)$, the rate equation for the system is given by

$$\frac{dC_{\text{ex}}}{dt} = -\frac{C_{\text{ex}}}{\tau} + B_1 E_1 w_1^{-2} \epsilon_1 (C - f C_{\text{ex}}) g_1(t), \quad (\text{A1})$$

where $C_{\text{gr}}(t)$ is the time-dependent concentration of the molecules in the ground state and $f = 1 + g_{\text{gr}}/g_{\text{ex}}$ represents the influences of degeneracy in the lower level (g_{gr} , ground state) and the upper level (g_{ex} , excited state). The solution of Eq. (A1) is

$$C_{\text{ex}}(t) = B_1 E_1 w_1^{-2} \epsilon_1 C \int_{-\infty}^t g(t') \times \exp\left[-\frac{t-t'}{\tau} - f B_1 E_1 w_1^{-2} \epsilon_1 \int_{t'}^t g(\xi) d\xi\right] dt'. \quad (\text{A2})$$

Equation (A2) can be evaluated with the given experimental

condition. The concentrations of C and C_{ex} in Eqs. (4) and (5) should be replaced to give the *Gain* and parameter χ in Eqs. (7), (8), and (9) under intense pump conditions.

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