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Development of Multiphoton Excitation Thermal Lens Spectroscopy for Label-Free and High-Sensitive Detection

高感度・無標識検出を指向した多光子励起熱レンズ分光法の開発

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1. Introduction

Most chemical species playing an important role in living organisms have absorption of light only in the UV region. However, conventional methods to detect these species haven't realized sufficient sensitivity yet. Therefore, UV laser excitation is desired for detection of various chemicals and biomolecules without labeling.

Photothermal spectroscopy including photothermal lens (PTL) method and photoacoustic (PA) methods is one of the most sensitive ways for detecting various chemical species[1]. Photothermal methods are based on heat generation in a light-illuminated medium *via* optical absorption and subsequent nonradiative relaxation. The signal magnitude is directly related to the amount of heat generated and temperature rise.

Deep-ultraviolet laser excitation at 213 nm has been attempted to develop a versatile PTL detection system for measuring dilute amino acids in liquid solutions. The PTL detection system was combined with a semi-micro HPLC for separation and detection. The sensitivity of PTL detector was 4-times higher than UV detector. However, two-photon absorption of the solvent could induce a high background signal and thus limit the analytical performance of this method.

In this work, PTL measurement under two-color pulsed laser excitation was examined. A PTL signal enhancement technique using multi-color excitation has been proposed to achieve higher sensitivity[2]. We aimed to excite target molecules from ground state (S_0) to excited state (S_1) with two-photon of two different wavelengths. It should be noted that wavelength-variable visible laser generated by optical parametric oscillator (OPO) was utilized as one of the excitation light source to detect target molecules selectively.

2. Experimental methods

A schematic illustration of the experimental setup for PTL detection by two-color two-photon pulsed laser excitation is shown in **Figure 1**.

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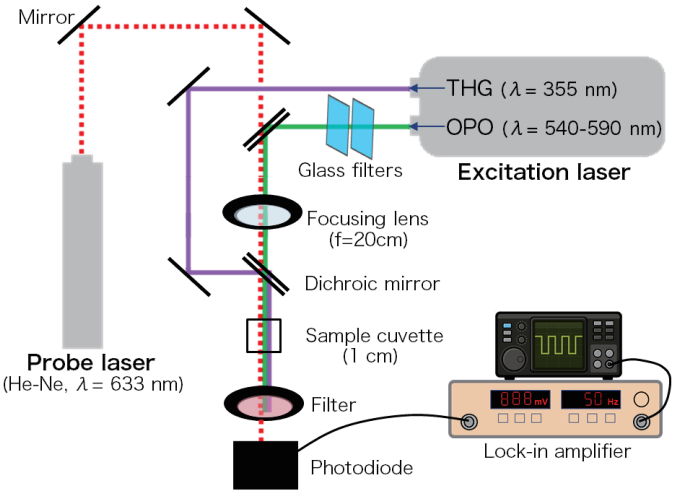

Figure. 1 Experimental setup for the PTL detection by two-color two-photon pulsed laser excitation. OPO: optical parametric oscillator (50 Hz, 3-5 ns).

An Nd:YAG laser system (NT230-50-SH/SF-2H-3H, EKSPLA) was used as the excitation beams. It comprises of the third harmonics generator (THG) and optical parametric oscillator (OPO) in a single device (repetition frequency, 50 Hz; pulse width, ~5 ns). Pulse timing of two excitation beams was synchronized at the sample cuvette. Photothermally generated heat in the sample solution was monitored with a He-Ne laser (LGK7654-08, LASOS, wavelength 632.8 nm) as the probe beam, which was focused with a focusing lens (f = 20 cm). The excitation beam of OPO was also focused with the focusing lens. These three beams were aligned coaxially by a dichroic mirror and irradiated into the solution. The excitation wavelength of OPO was varied from 540 to 590 nm. The quartz cuvette, which held a sample, was mounted on a 3-D stage. The light intensity at the center of the probe beam after passing through the sample solution was monitored with a photodiode (C4777, Hamamatsu), and the PTL signal intensity was measured with a lock-in amplifier. Beam power was adjusted with colored glass filters. Intensity was monitored in front of the sample cuvette. The samples chosen were amino acids (tryptophan and phenylalanine). These were diluted with acetonitrile/water = 80/20 volume %.

3. Results and Discussion

Absorption spectra of amino acids were measured. It was confirmed that the amino acids have optical absorptions only in the UV range.

Figure 2 shows PTL spectra of tryptophan and phenylalanine. These spectra are well-coincident with their absorption spectra within the wavelength range of 270 to 295 nm when a half of OPO wavelength is considered as wavelength of absorbed light. The excitation power dependence of the PTL signal was measured for a sample solution with a concentration of 1 mM. The signal intensity was proportional to the square of the OPO beam power. These results clearly show that spectra in **Figure 2** are single-color two-photon excitation PTL spectra. It is succesfullly demonstrated that nonresonant-resonant typed two-photon excitation is applicable to photothermal detection, although this demonstration is by single, not by two-color. Two-color two-photon absorption cross-section is not high for these species.

Generally, two-photon absorption is only observed in intense laser beams, particularly focused pulsed lasers, which generate a very high instantaneous photon density[3]. Focusing THG beam could be a dominant factor in a viewpoint of photon density. **Figure 3** shows PTL signal amplification factor at each OPO beam wavelength

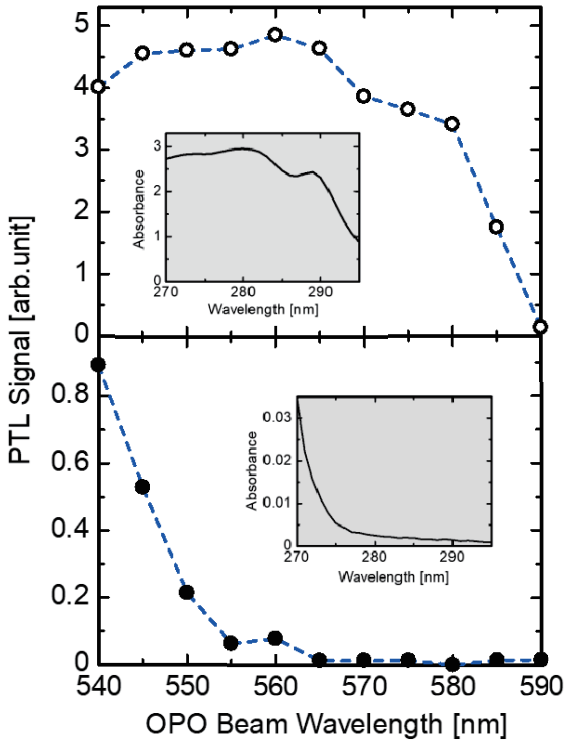

Figure. 2 PTL spectra of tryptophan and phenylalanine. Insets show absorption spectra.

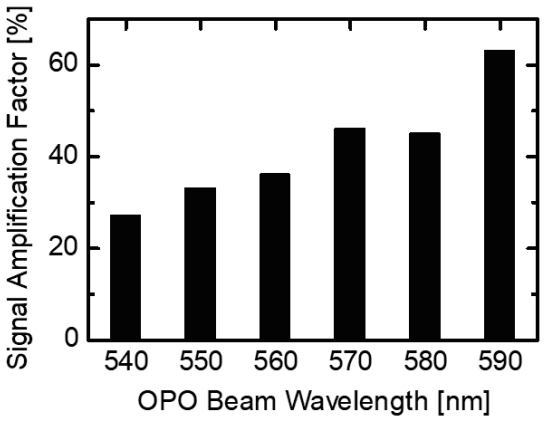

Figure. 3 PTL signal amplification factors at each OPO beam wavelength.

under simultaneous irradiation of focused OPO and focused THG beams. The amplification factor means a percentage of signal increase at the simultaneous irradiation with respect to the summation of signal intensities at each of single-color excitation. It rises with increasing excitation wavelength, which was clearly different from the trend seen in **Figure 2**. This results from two-color simultaneous excitation or amplification caused by transient absorption. By comparing **Figure 3** with the absorption spectrum of tryptophan in the wavelength range of 214~222 nm, corresponding to the summation of photon energy of THG and OPO beams, it is strongly suggested that two-color two-photon absorption occurs because these amplification factors have a correlation.

4. Conclusions

Two-photon excitation photothermal lens spectra were observed with a visible OPO laser beam and 355 nm beam. An amplification of PTL signal was demonstrated by two-color two-photon excitation. The signal was amplified up to 63 %, where sufficient focusing of excitation beams is essential. However, background signal was still observed at a high level. An enhancement of the solute signal is the key for further improvement in sensitivity.

References

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