

## Optimization for Ultra-violet Photothermal Image Measured with Reflection Objective

紫外光熱変換イメージングのための反射対物光学系の最適化

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### 1. Introduction

One of the ultimate goals of analytical biochemistry is to detect certain target molecules in a living cell and to observe molecular behavior *in-situ*. To observe living cells, microscopes are used. There are many types of microscope, such as optical, fluorescent, and electron, and each of them has merits and demerits, respectively. There is still no microscope which has high resolution and high selectivity and can observe cells *in-situ*. To achieve this goal, we have developed imaging methods by improving the performance of the photothermal spectroscopic method.

The thermal lens method, one of the photothermal methods, is suitable for observing substances in biological cells because it is based on the optical excitation and optical detection of photothermal effects, and it is easy to use in combination with optical microscopy<sup>1)</sup>.

Our research group has designed a photothermal microscope using the third harmonics of the Ti:sapphire laser as the excitation light source<sup>2)</sup> which enables direct comparison of photothermal and transmitted-light-intensity images to an image of the same object obtained with a complementary metal-oxide semiconductor image sensor (CMOS). With this system, a photothermal lens image of a single yeast cell was successfully obtained<sup>3)</sup>. In this system, reflection objective was used as the objective lens, for the use of a wide range of wavelength. However, there were some problematic points of image distortion in our system caused by the use of the reflection objective. we have verified these points and suggested the way to solve these problems.

### 2. Experimental methods

Fig. 1 shows a schematic illustration of the experimental setup for the ultraviolet-excitation microscopic photothermal lens imaging. The third harmonics of a Ti:sapphire laser was used as the excitation light source. A thermal lens was generated in a sample on a slide glass using the third harmonics (260 nm, < 3 mW). The thermal lens signal was probed with the fundamental

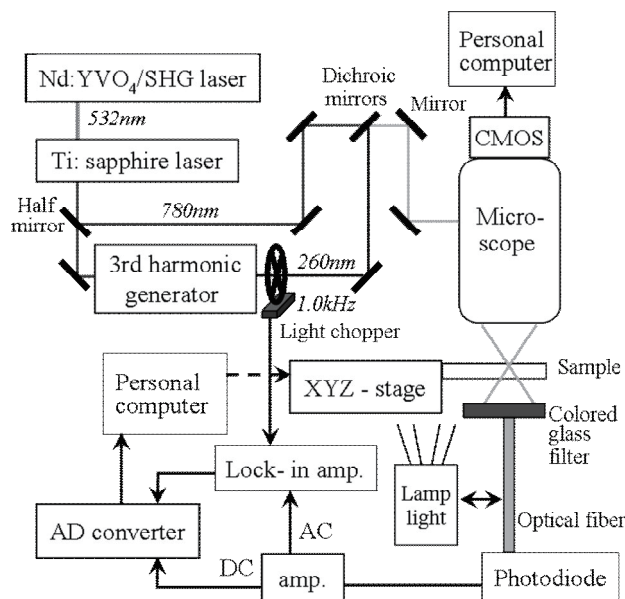


Fig. 1 Experimental setup for ultraviolet-laser excitation microscopic photothermal lens imaging. The photothermal lens signal is obtained from the AC output of the photodiode; the transmitted light intensity is monitored with DC output.

emission (780 nm, < 5 mW) of the Ti:sapphire laser. Both the excitation and probe laser beams were focused using a reflection objective lens (magnification,  $\times 20$ ; numerical aperture, 0.35). This reflection objective enabled the concurrent use of the infrared probe beam, ultraviolet excitation beam, and visible light for a CMOS camera. The excitation beam was intensity-modulated at 1.0 kHz with a mechanical light chopper. The light intensity of probe beam was monitored with a photodiode connected to an optical fiber. A colored glass filter set between the sample and the optical fiber, blocked the excitation beam. The thermal lens signal monitored with a lock-in amplifier was obtained from the AC output. The DC output of the photodiode was used to monitor transmitted light intensity.

The sample was set on an XYZ-mechanical stage that was computer-controlled using the laboratory-made software. Typically, a  $30 \times 30 \mu\text{m}^2$  and  $60 \times 60 \mu\text{m}^2$  areas were obtained in  $0.5 \mu\text{m}$  and  $1 \mu\text{m}$  scanning steps, respectively.

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Position-selective observation was achieved using a CMOS system attached to the photothermal microscope. For position-selective observation, the photodiode was replaced with a lamp light. The accuracy of the X-Y position was greater than 3  $\mu\text{m}$ . Yeast cells (4  $\mu\text{m}$   $\Phi$  in diameter) and cation exchange resin particles (11  $\mu\text{m}$   $\Phi$ , in diameter) were used as sample in the air on quartz plates under a quartz cover slip.

### 3. Results and Discussion

Though we have already successfully observed a single nonstained yeast cell with the photothermal microscope<sup>3)</sup>, there were some problematic points. Fig. 2 (a) (b) shows the photothermal images of the same cation exchange resin particle at the same position. In the experiments for these images, only the position of the optical fiber connected to detector was changed. Fig. 2 (c) (d) shows the transmitted light intensity images obtained in the same experiment as (a) (b), respectively. From these images, it can be seen that there are differences between the position of TL signals and the transmitted light intensity change, and that the differences depended on the position of the detector. Fig. 3 shows a schematic illustration of laser beam and optical fiber connected to detector when Fig. 2 was observed. Probe beam has a doughnut shape because of the reflection objective. Because the excitation beam waist size at the incident point to microscope is small, the spot size of excitation beam in Fig. 3 is small. When Fig. 2(b) was obtained, optical fiber was at the same position of excitation beam spot. The difference was the smallest with this condition.

Considering Figs. 2 and 3, the problems must have occurred by these laser configurations at the detecting point. From the results of experiments, we judged the optimum condition for imaging by photothermal microscope with the reflection objective: The spot size of the probe beam at the detecting point should be smaller and that of excitation should be larger. For this condition, the probe beam should be focused and the excitation beam should be expanded before the objective lens.

### 4. Conclusion

For observation of non-stained biological cells, we have developed an ultraviolet-excitation photothermal microscope. There were some problematic points of image distortion in the microscopic system caused by the use of the reflection objective. We have experimentally verified these points and suggested the way to solve problems as the optimum condition. With this condition, more accurate photothermal images will be obtained using our photothermal microscope.

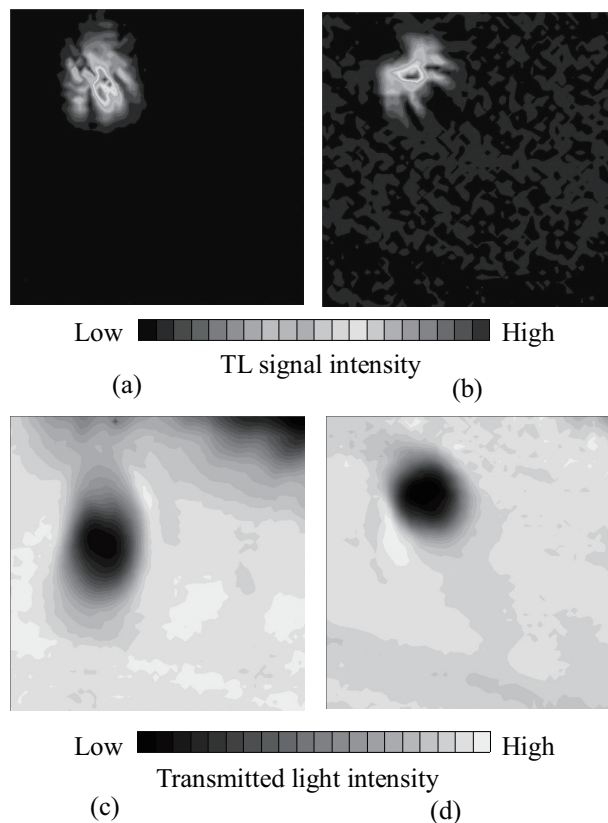


Fig. 2 (a), (b) Photothermal amplitude images of the same cation exchange resin particle. (c), (d) Transmitted light intensity images obtained in the same experiment as (a) and (b), respectively. Area size is  $60 \times 60 \mu\text{m}^2$ . These images were obtained for the same sample at the same condition except for the position of the detector.

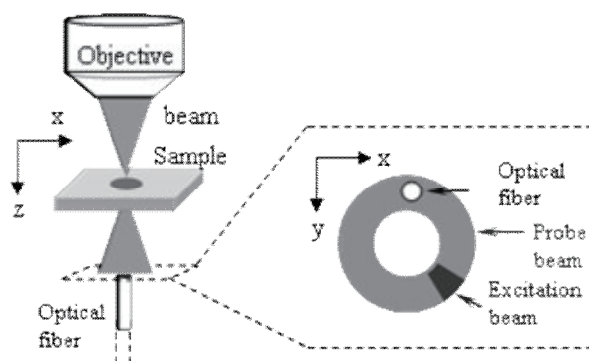


Fig. 3 Schematic illustration of the laser beams and the optical fiber connected to the detector.

### References

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