

Observation of Single Yeast Cells with Advanced Photothermal Lens Microscopes

新型熱レンズ顕微鏡による単一酵母菌の観察

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1. Introduction

In the biological field, microscopy has played great roles to discover new insights of biological cells. Nowadays, fluorescence microscope has been used widely in this field because of its good resolution and selectivity to chemical species. Of course, fluorescence microscope require samples to fluoresce. However, There are many biologically important chemicals which are nonfluorescent. If one want to observe a sample which consists of only nonfluorescent chemicals, fluorescence staining will be used in many cases. Though the fluorescence staining is obviously great technique, there is no guarantee that an image of a sample aquired with the staining is a plain image of the sample.

To overcome this difficulty, we have developed thermal lens UV microscopic system which uses photothermal spectroscopy for contrast-generation. Photothermal spectroscopy is one of the most sensitive detection methods for nonfluorescent chemical species in a liquid solution. 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 effects¹⁾.

Photothermal spectroscopy requires the optical absorption of sample materials, so we chose UV laser beam as an excitation beam for label free imaging of biological cells.

With the photothermal lens UV imaging system developed, a photothermal lens image of a single nonstained yeast cell has been observed successfully²⁾, in which a reflection objective lens is used for focusing an excitation laser beam at 260 nm and a probe laser beam at 780 nm. The thermal lens image distortion observed with that system was analyzed and the reflection-objective-collinear (roco) setup is proposed. It has been demonstrated that the roco-system removes this distortion for a cation exchange particle^{3), 4)}.

This time, we demonstrate imaging of single yeast cells with the advanced photothermal lens microscope.

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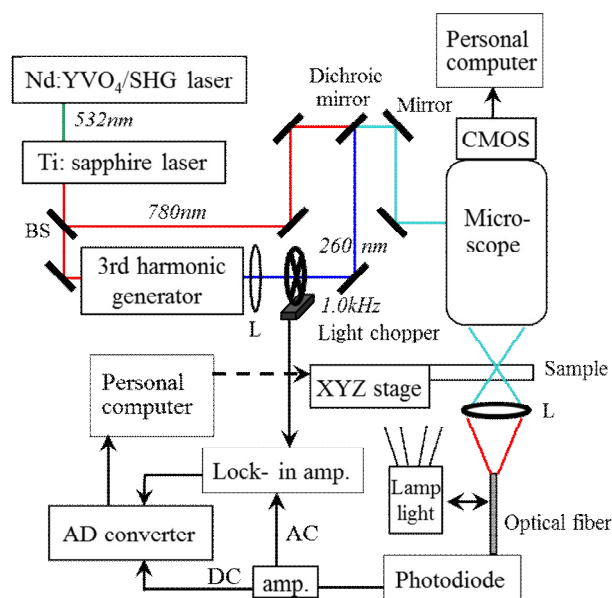


Fig. 1 Experimental setup for the photothermal lens imaging system.

L, lens; BM, beam splitter; CMOS, complementary metal-oxide semiconductor image sensor; and amp., amplifier.

2. Experimental methods

Figure 1 shows a schematic illustration of the experimental setup for the photothermal lens UV imaging system. The third harmonics (260 nm) of a Ti:sapphire laser was used as the excitation beam source. A thermal lens was generated in a sample. The thermal lens signal was probed with the fundamental emission (780 nm) of the Ti:sapphire laser. Both the excitation and probe laser beams were focused using a reflection objective lens (magnification, $\times 20$; numerical aperture, 0.38). This reflection objective has enabled the concurrent use of the infrared probe beam, ultraviolet excitation beam, and visible light for an optical image sensor. The excitation beam was expanded with a lens and 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. This optical fiber played a role as a pinhole. A lens was set between the sample and the optical fiber,

focusing the probe beam at one point and cutting 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. Position-selective observation was achieved using the optical image sensor. For position-selective observation, the photodiode was replaced with a lamp light. The accuracy of the X-Y position was greater than $3\ \mu\text{m}$. Polystyrene particles ($7\ \mu\text{m}$ Φ in diameter) were used as a test sample. As a biological sample, yeast cells were used.

3. Results and discussion

Figure 2 shows the photothermal lens image of a single polystyrene particle ($7\ \mu\text{m}$ Φ in diameter) and its optical image. The pixel count is 60×60 and the area size is $30 \times 30\ \mu\text{m}^2$. As shown in the optical image, there is only one particle in the scanned area and thus we have successfully obtained a thermal lens signal image of a single polystyrene particle. We also scanned same area without excitation laser beam and confirmed that there was no signal. Because the position of detection is fixed at one point, there is no need to be afraid the distortion problem which our old system had. We can easily observe similar image repeatedly.

Figure 3 shows the photothermal lens image of two single nonstained yeast cells and its optical image. The pixel count is 60×60 and the area size is $60 \times 60\ \mu\text{m}^2$. The positional relationship of two yeast cells seen in the optical image (b) is apparently reflected in the photothermal lens image (a). We can say that we have succeeded to observe single nonstained yeast cells with our advanced photothermal lens microscope.

4. Conclusion

We have successfully obtained photothermal lens signal images of nonstained single yeast cells with advanced photothermal lens microscope. With this system, there is no need to be afraid the distortion which was caused by position selectivity of detector. Though we used Ti:sapphire laser beam as the probe beam, we can also use He-Ne laser beam as we have reported before⁴⁾. We will show further advance in the photothermal lens microscope for visibly transparent biological cell observation.

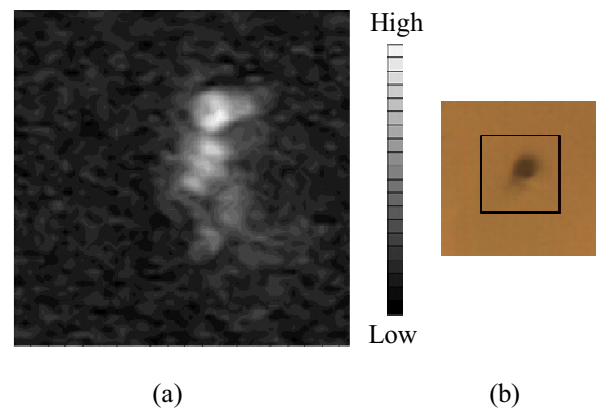


Fig. 2 (a) Photothermal lens image of a polystyrene particle ($7\ \mu\text{m}$ Φ in diameter). Scanned area size is $30 \times 30\ \mu\text{m}^2$. Right bar shows the intensity of thermal lens signal. (b) Optical image of the scanned sample. The size of the square in this image is $30 \times 30\ \mu\text{m}^2$

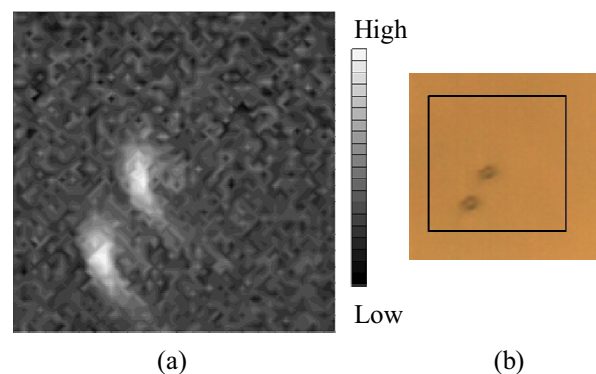


Fig. 3 (a) Photothermal lens image of two single nonstained yeast cells. Scanned area size is $60 \times 60\ \mu\text{m}^2$. Right bar shows the intensity of thermal lens signal. (b) Optical image of the scanned sample. The size of the square in this image is $60 \times 60\ \mu\text{m}^2$

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