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Development of Multi-color Excitation Photothermal Lens Microscope

多色励起熱レンズ顕微鏡の開発

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1. Introduction

It is effective for acquirement of a new insight in biochemistry to improve the performances of microscopes or develop a novel microscope – needless to say, there had been many discoveries with the development of the microscopes. Nowadays, fluorescence microscope has been used widely in this field because of its good resolution and selectivity to chemical species. Of course, fluorescence microscopes require samples to fluoresce. However, there are many biologically important chemicals which are nonfluorescent unfortunately.

To overcome this difficulty, we have been studying photothermal lens imaging 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 photothermal 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[1)]

In our previous research, we used UV laser beam as the excitation and a reflective objective for focusing[2)-4)]. Though we have succeeded to observe a single yeast cell with our system, we also found that it is difficult to find a best beam configuration with a reflective objective for a sample which size is smaller than 1 μm in diameter.

In this research, we developed the multi-color excitation photothermal lens microscope with conventional objective lens. We investigated the excitation wavelength dependence of photothermal lens signal images through the observation of aggregated gold nano particles. The absorption spectrum of gold nano particles depend on the size of the particles, size sensitive imaging will be expectied with the multi-color excitation photothermal lens microscope.

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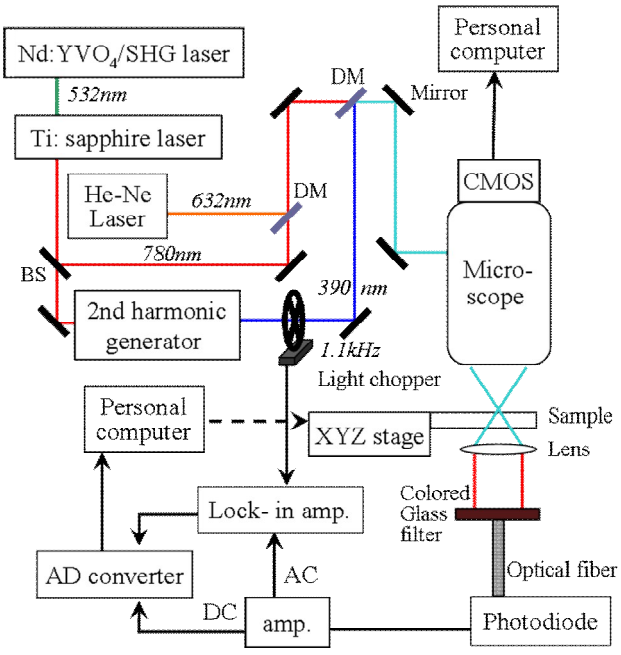

Fig. 1 Experimental setup for the multi-color excitation photothermal lens imaging system.
DM, dichroic mirror; BM, beam splitter; CMOS, complementary metal-oxide semiconductor image sensor; and amp., amplifier.
The light chopper was moved in the optical path of the He-Ne laser beam when He-Ne laser was used as the excitation beam.

2. Experimental methods

Figure 1 shows a schematic illustration of the experimental setup for the multi-color excitation photothermal lens imaging system. The second harmonics (390 nm) of a Ti:sapphire laser or He-Ne laser beam (632 nm) was used as the excitation light source. 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 an objective lens (magnification, ×20; numerical aperture, 0.40). The excitation beam was intensity-modulated at 1.1 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, gathering the probe beam to the fiber. The excitation beam was cut with the colored glass filter before the optical fiber. 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 a 3D-mechanical stage that was computer-controlled using the laboratory-made software. Position-selective observation was achieved using the optical image sensor. Aggregated gold nano particles which have broad absorption band were selected as a test sample.

3. Results and discussion

Figure 2 (a) and (b) show the photothermal lens imaging results of aggregated gold nano particles. The pixel count is 100 × 100 and the area size is 30 × 30 μm^2. The excitation beam was (a) the second harmonics (390 nm) of a Ti:sapphire laser and (b) He-Ne laser beam (632 nm), respectively. Fig.2 (c) is the transmitted light intensity image of the same area as (a) and (b). This image was obtained simultaneously with (a). Fig.2 (d) shows the dark-field image of the scanned area. There is no particle in the black area of this image. The size of this image is also 30 × 30 μm^2.

The photothermal lens signals in Fig.2 (a) and (b) were obviously acquired from the gold nano particles because the positions of signals in these images well correspond to the optical image (d). As shown in Fig.2 (c), light intensity was changed over the wider area than the area particles were located in (d). This is because the spot size of probe beam. These results show that the resolution of photothermal lens microscope is pretty well compared with the probe beam absorption.

The comparison of the images (a), (b) shows that the more detailed structure of aggregated particles is observed with shorter excitation wavelength.

4. Conclusion

We developed the multi-color excitation photothermal lens microscope and demonstrated the excitation wavelength dependence of the photothermal lens signal images via the imaging of the aggregated gold nano particles. With the sample which has narrow absorption band, there will be remarkable change in the images with different excitation wavelengths. As 632nm light absorption of gold depends on Aggregated the particle size, particle size sensitive imaging will be expected with multi-color excitation photothermal lens microscope.

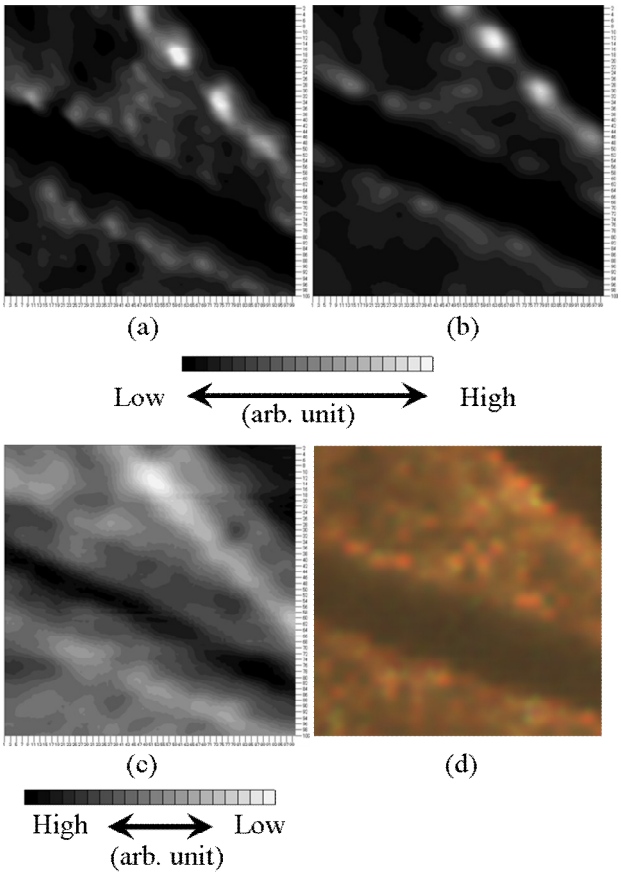

Fig. 2 (a), (b) Photothermal lens images of aggregated gold nano particles with the different excitation wavelengths. Scanned area size is 30 × 30 μm^2 and the pixel count is 100 × 100. The bar under images shows the intensity of thermal lens signal.
The excitation beam was (a) the second harmonics (390 nm) of a Ti:sapphire laser and (b) He-Ne laser beam (632 nm).
(c) Transmitted light intensity image of aggregated gold nano particles which was observed simultaneously with (a). Scanned area, size, and pixel count were same as (a) and (b). The bar under images shows the transmitted light intensity.
(d) Scattered light image of the scanned area obtained with the CMOS image sensor. The size of this image is 30 × 30 μm^2.

Acknowledgment

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