1Pb-20

Proceedings of Symposium on Ultrasonic Electronics, Vol. 31 (2010) pp. 181-182
6-8 December, 2010

Development of Photothermal Lens UV Imaging Method for Living Cells

生細胞観察のための紫外熱レンズイメージング法の開発

Noriyuki Fujii[1, 2‡] and Akira Harata[1]
([1]Dept. of Mol. and Mat. Sci., Kyushu Univ., [2]JSPS Research Fellow)
藤井 宣行[1, 2‡], 原田 明[1] ([1]九大院 総理工, [2]日本学術振興会特別研究員DC)

1. Introduction

It is effective for acquirement of a new insight in biochemistry to improve the performances of microscopes or develop a novel microscope. Though fluorescence microscope is a powerful tool for biological imaging with which the distributions of fluorescent substances are observed with ultrahigh sensitivity up to the single-molecule detection level, it is not suitable for the *in-situ* observation of nonfluorescent samples without fluorescent labeling. Because of this, it is desirable to develop ultrasensitive label-free imaging methods for nonfluorescent compounds in and around a living cell.

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[1)].

We have developed a photothermal lens UV imaging method for observing chemical compounds in biological cells, 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 system enables direct comparison of photothermal and transmitted-light-intensity images to an image of the same object obtained with an optical image sensor. We have already reported a photothermal lens image of a single nonstained yeast cell successfully with this system [3)]. To improve the method to obtain accurate photothermal lens UV images, we investigated how the position of the signal ditection affects photothermal lens UV images and made the effects of optical paths of laser beams on image distortion clear.

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 light 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, ×20; numerical aperture, 0.35). This reflection objective has enabled the concurrent use of the infrared probe beam, ultraviolet excitation beam, and visible light for a complementary metal-oxide semiconductor image sensor (CMOS) camera. The excitation beam was expanded with a lens to fix the optical path under the objective 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 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.

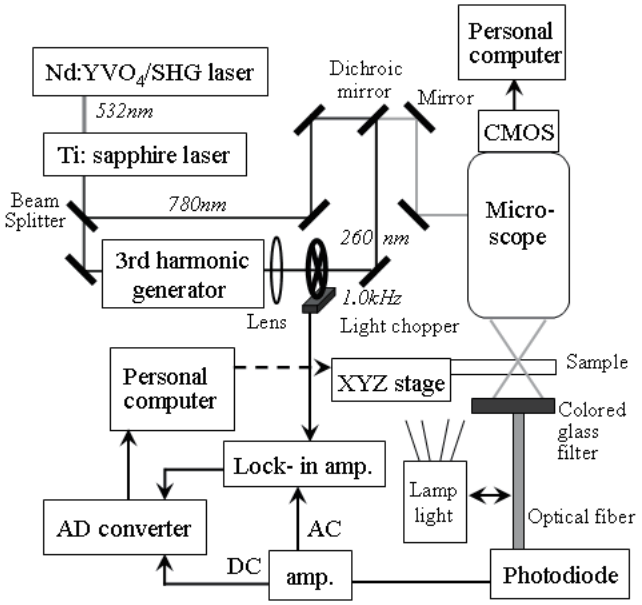

Fig. 1 Experimental setup for the photothermal lens ultraviolet imaging system. The photothermal lens signal is obtained from the AC output of the photodiode; the transmitted light intensity is monitored with DC output.

The sample was set on an XYZ-mechanical

hr-n-fujii@mms.kyushu-u.ac.jp

stage that was computer-controlled using the laboratory-made software. Typically, 60 × 60 μm^2 areas were obtained in 1 μm scanning steps. Position-selective observation was achieved using the CMOS system. For position-selective observation, the photodiode was replaced with a lamp light. The accuracy of the X-Y position was greater than 3 μm. Cation exchange resin particles (11 μm Φ in diameter) were used as sample in the air on quartz plates under a quartz cover slip to investigate the optimum conditions for the system.

3. Results and Discussion

Figure 2 shows the photothermal lens UV images of the same cation exchange resin particle at the same position. In the experiments for these images, there was no change in the imaging conditions containing scanned area, except for the position of the optical fiber connected to detector. Unlike the previous works[4], these images reflect only the effect of the position of the detecting point, shutting down the effect of the light path of the excitation beam. Because the optical fiber limited the probe beam detected, the positional change produced the same effect as the change in the optical path of the probe beam. Thus, these results shows the fact that the change of optical paths affect the photothermal lens UV images obtained with our system, and that there is a need for a method to fix the optical path of the probe beam in order to obtain an accurate photothermal UV image with this system.

Figure 3 shows the schematic illustration of a advanced detection system to obtain accurate photothermal UV images. With the condenser lens before optical fiber, all optical paths of the probe beam are focused into the optical fiber. We have already confirmed that the thermal lens signals could be obtained with this detection system.

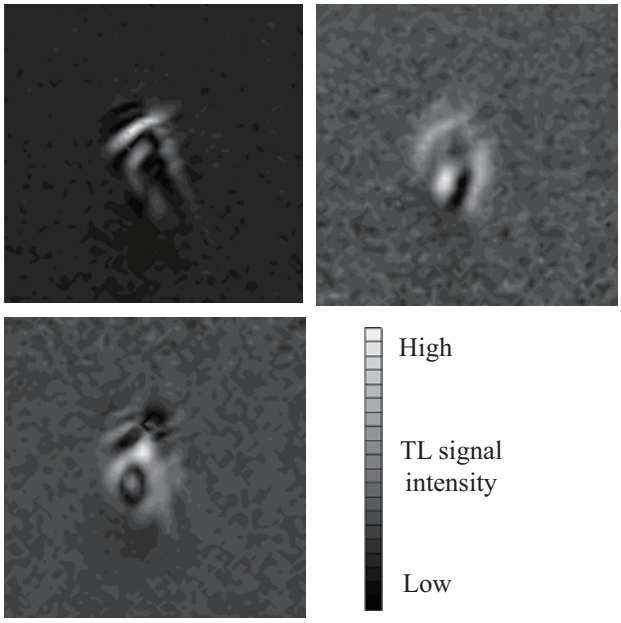

Fig. 2 Photothermal lens images of the a cation exchange resin particle.
These images were obtained for the fixed sample under the fixed conditions except for the position of the detector.

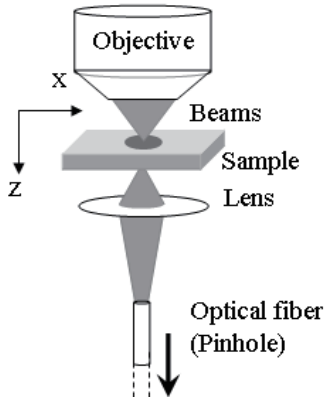

Fig. 3 Schematic illustration of the advanced detection system. The optical path of probe beam is uniquely settled in this configuration.

4. Conclusion

We have investigated how the position of the signal detection affects photothermal lens UV images for further sensitive and accurate *in-situ* observation of single nonstained living cells. Here, we made it clear that the optical paths of laser beams affect the thermal lens UV images and suggested the advanced detection system. With the advanced detection system, in which the optical paths of the laser beams are uniquely settled, accurate thermal lens UV images will be obtained.

Acknowledgment
This work was supported by Grant-in-Aid for JSPS Fellows.

References

1. A. Harata, in 'Nano Biophotonics: Science and Technology', Elsevier BV, pp. 73-92 (2007)
2. A. Harata, T. Matuda and S. Hirashima: Jpn. J. Appl. Phys. **46** (2007) 4561
3. N. Fujii and A. Harata: Jpn. J. Appl. Phys. **48** (2009) 07GC09
4. N. Fujii and A. Harata: Proc. of the 30th Symp. on ultrasonic electronics, 2009, pp. 85-86