PHOTOTHERMAL IMAGING OF ABSORBANCE DISTRIBUTION WITH SYNCHRONOUS CCD DETECTION METHOD

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ABSTRACT

In this paper, we have developed a novel photothermal microscopy to imaging absorbance distribution with a synchronous CCD detection method. This method can measure several-tens-of-micrometers square area simultaneously, and the synchronous detection separates the absorbance image from the background bright-field images.

KEYWORDS: Imaging, Absorbance, Photothermal spectroscopy

INTRODUCTION

Thermal lens microscope (TLM), one of photothermal spectroscopies, can measure very little absorbance with short optical length, and ultrahigh sensitivity of TLM is demonstrated in various MicroTAS applications [1]. By scanning the laser spot, TLM can also measure absorbance distribution in a sample under a microscope. For example, distribution of cytochrome c during apoptosis process of single cell was monitored [2]. However, conventional TLM imaging method required the laser-spot step-scanning and was time-consumable, where an hour was required for 30-µm-square scan. In this paper, synchronous CCD detection method has been developed and its characteristics have been demonstrated.

CONCEPTION

Figure 1a shows conventional scanning TLM. When the sample absorbs the excitation light, the absorbed energy is released to the surrounding medium as thermal energy to generate temperature gradient around the excitation beam. The temperature increase corresponds to decrease of refractive index and the temperature gradient acts as transient optical lens, which is called as thermal lens effect. The thermal lens effect deflects the probe beam locus to change the probe beam intensity after the pinhole. In order to reduce backgrounds during the probe intensity change, amplitude of the excitation is modulated and modulated component of the probe beam is synchronously detected by a lock-in amplifier.

Figure 1b shows concept of the novel photothermal microscopy. The excitation modulated with a frequency of $f_{\text{ex}}$ and the probe beams irradiate wider area of the sample, typically 80 µm square. The intensity of the probe beam is detected by a CCD camera.
Figure 1. (a) Conventional scanning thermal lens microscope (TLM). The excitation and probe beams are tightly focused, and the spot is scanned to obtain the analyte image. (b) Concept of novel photothermal microscope. The excitation and probe beams irradiate wider area of the sample. The image of analyte distribution is obtained by CCD camera simultaneously.

EXPERIMENTAL

To reduce the background brightfield image, the signal from each pixel of CCD should be detected synchronously, but no such device exists. Figure 2 shows scheme of synchronous CCD detection. Here, the probe beam is modulated with $f_{ex} + 5$ Hz and the beat signal (5 Hz) between the photothermal effect ($f_{ex}$) and the probe modulation ($f_{ex} + 5$ Hz) is detected by CCD working at 20 Hz. Four serial images make a unit data set. By calculating the data set, we can obtain a DC and AC image. While DC image corresponds to background one, AC image shows synchronous image with the photothermal effect ($f_{ex}$). Thus, the photothermal image can be obtained by ordinary scientific CCD system.

Figure 2. Scheme of synchronous CCD detection. The probe beam is modulated with $f_{ex} + 5$ Hz and the beat signal (5 Hz) between the photothermal effect ($f_{ex}$) and the probe modulation ($f_{ex} + 5$ Hz) is detected by CCD working at 20 Hz. Four serial images make a unit data set. By calculating the data set, we can obtain a DC and AC image.
RESULTS AND DISCUSSION

The excitation (532 nm) and probe (633 nm) lasers were modulated with an acousto-optic modulator (AOM) with $f_{\text{ex}}$ and $f_{\text{ex}} + 5$ Hz, respectively. CCD images were recorded in the computer and calculated by a lab-made program. Figure 3a shows an illustration of sample microstructure. The dye solution was filled in 10-$\mu$m-depth triangle well. Figure 3b shows DC image (normal micrograph) and Figure 3c shows AC photothermal image. Clear photothermal signal due to absorbance distribution was imaged. The detection limit is $1.5 \times 10^{-5}$ Abs., which is 2-order superior to usual microscope observation. The acquisition time is only 20 s while that of conventional scanning TLM requires 1 hour.

Figure 3 (a) Illustration of sample microstructure. The dye solution was filled in 10-$\mu$m-depth triangle well. (b) DC image (normal micrograph). (c) AC photothermal image. The white contrast corresponds to strong light absorption. The acquisition time of these images is only 20 s while that of conventional TLM was 1 hour.

REFERENCES

