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Improvement of signal-to-noise ratio in photothermal microscopy by optimizing detection aperture

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ABSTRACT

We present a method for improving the signal-to-noise ratio in photothermal microscopy by optimizing the detection aperture in the spatially segmented balanced detection scheme. Experimental studies show a 1.44-fold improvement in the signal-to-noise ratio compared with the previous scheme. Consequently, the ratio is 9.6 times larger than that in conventional detection. The proposed method is implemented in a laser scanning photothermal microscope using low-cost compact laser diodes as the light source.

1. Introduction

Photothermal (PT) microscopy is an efficient method for observing non-fluorescent chromophores with a high degree of spatial resolution and single-molecule detection sensitivity. Particularly, this method is useful to visualize the distribution of endogenous chromoproteins in biological tissues without labeling. It has been applied for imaging cytochromes in mitochondria [1–4], hemoglobin in blood vessels [5], and melanin pigments in skin cancer [3,6]. These chromophores absorb light but do not fluoresce efficiently because of their fast non-radiative decay. Hence, microscopy techniques that can detect non-fluorescent molecules are highly desirable in biological science applications.

PT microscopy is a form of pump and probe microscopy [7] in which two laser beams with different wavelengths for pumping and probing are incident on the sample through a focusing lens. The pump beam increases the temperature, ΔT, around the focal point of the optical absorbing sample, which results in variations in the local refractive index and induces the deflection of the probe beam. To implement high-sensitivity imaging in pump-probe microscopy, it is important to circumvent the intensity noise of the probe beam. In stimulated emission and stimulated Raman microscopy, the intensity of the pump beam is modulated at high frequency (>1 MHz) to improve the signal-to-noise ratio (SNR) because the laser intensity noise of a solid state laser used for probing occurs primarily at low frequencies (from kilohertz to DC) in the form of $1/f$ noise [7–10]. However, in PT microscopy, the high-frequency modulation scheme reduces signal intensity because the time response of the PT signal is, in principle, determined by heat conductivity and is decreased by half at ~1 MHz for a tightly focused laser beam [11,12]. Moreover, the high-frequency modulation scheme cannot circumvent the high-frequency laser noise that usually presents in the laser diodes (LDs) and amplified fiber lasers [13].

In a previous study, we proposed a spatially segmented balanced detection (SBD) scheme to improve the SNR [14,15]. In the SBD scheme, the inner and outer parts of the transmitted probe beam are separated and detected by a balanced detector. We found the scheme to produce an up to twofold improvement in the PT signal while also canceling the intensity noise of the probe beam. We incorporated the SBD scheme in a laser scanning PT microscope using a bifurcated fiber bundle, and demonstrated the 3D visualization of endogenous mouse brain signals [14]. The major advantage of this method is that high-sensitivity imaging can be conducted using low-cost compact LDs.

SNR improvement is crucial to achieving fast imaging because the SNR is proportional to the square root of measurement time (time constant of the lock-in amplifier) [16,17]. The PT signal increases with pump power, but lower pump power is preferable to avoid photo and/or thermal damage to the sample. PT imaging at fast time scales has potential applications in live cell imaging and the tracking of biomolecular transport. Furthermore, one of the advantages of PT microscopy is its optical sectioning capability, as in confocal microscopy [3,18], as fast measurement is needed to acquire a stack of images at different focal plane depths within a limited amount of time.

In this study, we report on the optimization of the SBD scheme to further improve the SNR by optimizing the detection aperture using an annular light block. Another advantage of the use of an annular light block and possibilities for further SNR improvement are discussed.

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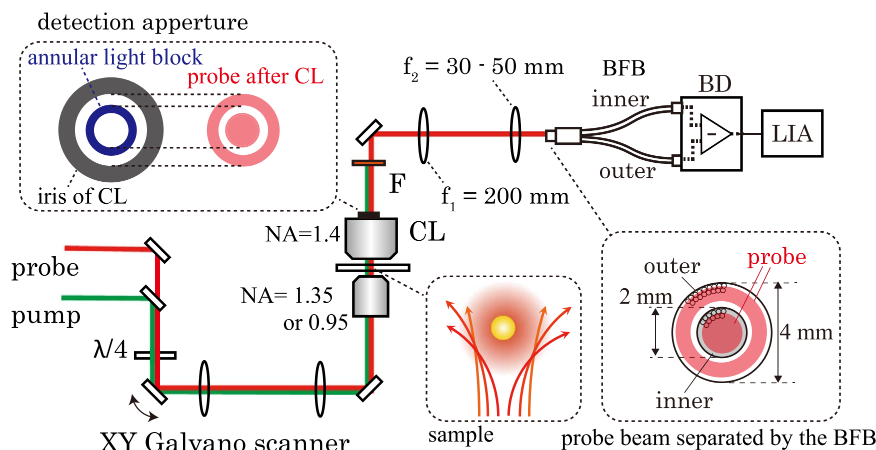

Fig. 1. Schematic illustration of a photothermal microscope with spatially segmented balanced detection. An annular light block is placed on the pupil plane of the condenser lens (CL) to optimize the detection aperture. OL: objective lens, F: filter, BFB: bifurcated fiber bundle, BD: balanced detector, LIA: lock-in amplifier.

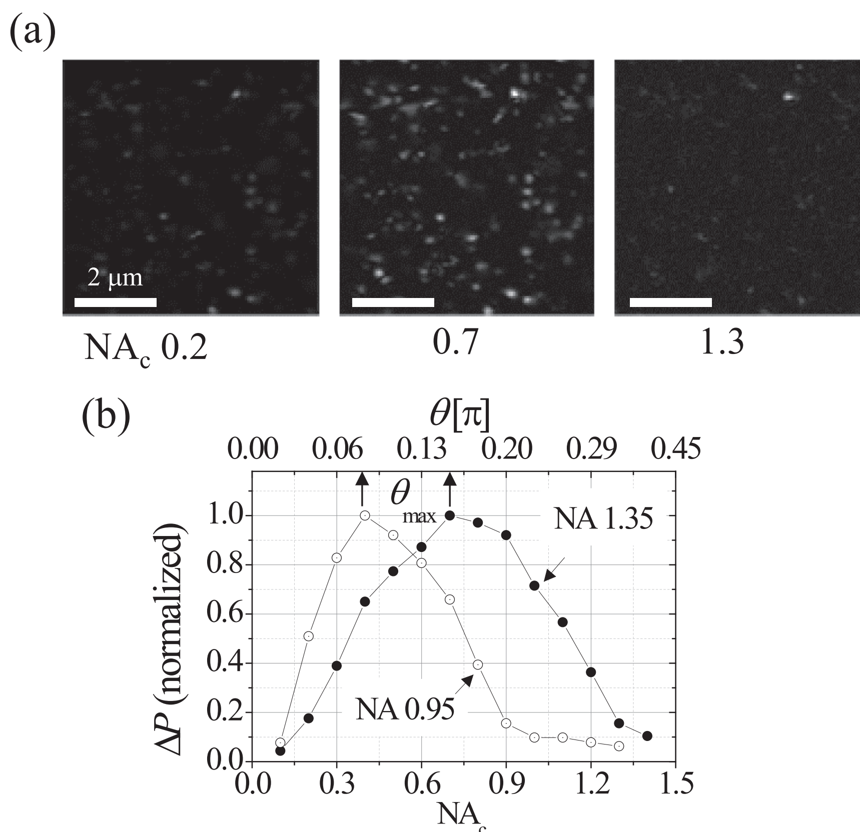

Fig. 2. Angular dependence of the photothermal signal measured with the conventional setup by changing collection aperture NA_c. (a) Photothermal images of gold nanoparticles dispersed in PVA with an NA_c of 0.2, 0.7, and 1.3. The NA of the focusing objective lens is 1.35. Image acquisition time was 7 s with 300×300 pixels. (b) Relation between photothermal signal intensity ΔP and NA_c with an NA of 0.95 (open circles) and 1.35 (filled circles). ΔP is obtained by integrating the signal over all pixels and subtracting the background noise. θ_{max} is defined as the angle at which ΔP exhibits the maximum.

2. Experiment

2.1. Experimental setup

An inverted laser scanning PT microscopy was constructed based on our previous setup (Fig. 1) [14], with 520-nm (THORLABS L520P120) and 640-nm (THORLABS HL6385DG) LDs used for pumping and probing, respectively. The pump beam was typically modulated at 140 kHz. The combined pump and probe beam was directed toward an XY Galvano scanner (GSI VM500PULS), and focused on a sample through an oil immersion objective lens (Olympus UPLSAPO60X) [numerical aperture (NA)=1.35] or a dry objective lens (Olympus UPLSAPO40X, NA =0.95).

The transmitted probe beam was collected by an oil immersion condenser lens, with NA=1.4 (Olympus U-AAC). The condenser lens is equipped with an iris diaphragm to change the collection aperture of the transmitted probe beam. The effective aperture or the angle was calculated by reading the scale of the iris. Furthermore, an annular light block was placed on the back pupil of the condenser lens to ensure that the cross-section of the transmitted probe beam formed a circle and a concentric annulus. The annular light block was cut out from a black acrylic plate using a laser cutter.

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