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Experimental evaluation of temperature increase and associated detection sensitivity in shot noise-limited photothermal microscopy

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ABSTRACT

Photothermal microscopy is useful for visualizing non-fluorescent chromophores in a light-scattering medium. However, it remains a major challenge to enhance sensitivity while reducing thermal damage to samples, particularly for biological tissues. In this study, a highly sensitive laser scanning photothermal microscope was constructed by implementing a low-noise balanced photodetector into a spatially segmented balanced detection which was proposed and demonstrated to be useful for high sensitivity detection in our previous studies. We confirmed that the noise level was only 1.05-fold greater than the shot noise. Temperature increase and the detection sensitivity for this parameter were evaluated by observing the motion of individual 20-nm-sized gold nanoparticles embedded in agarose gel in response to incident light at varied intensities. We found that the nanoparticles remained trapped in the gel structure at a low pump power and it exhibits random motion as increase in the pump power because local temperature of agarose gel around the nanoparticle reached the melting point of 338 K. The peak SNR is 290 at about the melting point with an integration time of 10 μs per pixel. On an assumption that the SNR is proportional to the temperature increase, the estimated limit of detection of a temperature increase at the nanoparticle surface, which is defined as the ratio of temperature increase to the SNR, ranges from 0.1 to 0.2 K. Additionally, findings from single carbon nanotube imaging suggest that a larger or longer light absorber would improve the trade-off relationship between the signal-to-noise ratio and temperature increase.

1. Introduction

Photothermal (PT) microscopy can detect non-fluorescent chromophores with a high degree of spatial resolution and detection sensitivity at the single-molecule level in a light scattering medium [1–4]. This modality has been used to visualize the distributions of endogenous chromophores in biological specimens such as hemoproteins in mitochondria [5–7], red blood cells in microvascular networks [8], and melanin pigments in skin cancers [7,9]. Furthermore, gold nanoparticle-based labeling techniques have been used to identify biomolecules in cellular imaging [10–13]. Non-fluorescent chromophores absorb light but do not fluoresce efficiently because they exhibit rapid non-radiative decay. Hence, PT imaging is inherently less affected by photobleaching, which is a problematic issue in fluorescence imaging. Although fluorescence-based techniques are currently most popular for cellular imaging, other microscopy techniques that would further expand our knowledge of biological processes are strongly desired.

PT microscopy is a form of pump-and-probe microscopy in which two laser beams with different wavelengths for pumping and probing are incident on a sample through a focusing lens [14,15]. The pump beam increases the temperature around the focal point of the light-absorbing sample, which causes variations in the local refractive index and induces deflection of the probe beam (i.e., nano-lensing) [16]. As the intensity of the pump beam is modulated, changes in the refractive index are detected as changes in probe beam transmissivity or reflectivity via a lock-in detection scheme. The focused laser beams are scanned sequentially in a point-by-point manner, and the resulting pixel information is assembled into an image.

The PT signal increases with the pump power because the temperature increase and consequent refractive index variation is proportional to the pump power. However, a lower pump power is preferable, as it reduces thermal and photo damage to the sample. Because the temperature coefficient of refractive index variations is small (i.e., $\partial n/\partial T \sim -1 \times 10^{-4}$/K for water), the relative change in probe beam intensity is weak (typically, $\sim 10^{-4}$). Therefore, an improved signal-to-noise ratio (SNR) is crucial to avoid irradiation damage to the sample. An improved SNR is also important for rapid imaging because

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the SNR is proportional to the square root of the integration time [17].

High-sensitivity imaging requires an enhanced signal intensity and reduced noise floor in the experimental system. In our previous work, we proposed a spatially segmented balanced detection (SBD) scheme to improve the SNR for transmission PT microscopy [18,19]. In this SBD scheme, the inner and outer parts of the transmitted probe beam are separated and detected by a balanced detector. We found that this scheme improved the PT signal by as much as two-fold while also canceling the probe beam intensity noise. The SBD scheme was subsequently incorporated into a laser-scanning PT microscope using a bifurcated fiber bundle, and this system was shown to provide a 3D visualization of endogenous chromophores in the mouse brain [19].

Thermal damage is a critical issue in PT imaging, especially for live-cell imaging because heat stimulation denature biological molecules and alter cellular functions. Previous reports have estimated temperature increases of 15 K on the surfaces of 10-nm-sized individual gold nanoparticles in live cells with an SNR of 20 and an integration time of 10 ms [10]. According to another report, this surface temperature increase was reduced to 0.1 K when glycerol (large $\partial n/\partial T$) was used as the medium [17]. However, these temperature values were determined via calculations [4]. To date, no study has attempted to confirm these temperature increases experimentally in the direct context of PT imaging. To further expand the performance and applications of PT imaging, therefore, temperature increases and possible thermal damage to samples must be investigated.

In this study, a low-noise balanced photodetector optimized for the SBD scheme was constructed with the aim of enhancing the SNR to the limit of the shot noise. The temperature increase and the detection sensitivity for this parameter were then evaluated by observing the motion of individual gold nanoparticles and carbon nanotubes (CNTs) embedded in agarose gel.

2. Experiment

2.1. Experimental setup

A high-sensitivity laser-scanning PT microscope was constructed based on our previous setup (Fig. 1) [19,20]. Here, a single-frequency laser diode with a center wavelength of 780 nm (THORLABS, LP785-SAV50) was used for probing. Near-infrared light is usually suitable for probing because it allows a maximum penetration depth of biological tissues with minimum absorption. A 520-nm laser diode (OSLAM PL-520-B1) was used for pumping. The intensity of the pump beam was modulated at a frequency of 500 kHz by modulating the injection current. The combined pump and probe beam was directed toward an XY Galvano scanner and focused on a sample through an oil immersion objective lens (Olympus UPLSAPO60XO) [numerical aperture (NA) = 1.35]. A variable neutral-density filter was used to control pump power.

The transmitted probe beam was collected by an oil immersion condenser lens. Spatially segmented balanced detection occurred via a bifurcated fiber bundle, which separated the inner and outer parts of the transmitted probe beam. The separated probe beams were then directed toward a balanced photodetector. An annular light block with inner and outer diameters of 12 and 17 mm, respectively, was placed on the back pupil of the condenser lens to achieve a 1.44-fold improvement in the SNR [20].

Although the maximum output power of the probe laser was 40 mW, the probe power at each port of the balanced photodetector was 0.43 mW. Although we used a commercial large-area balanced photodetector in a previous study, but probe power was insufficient for high-sensitivity detection. For this study, a low-noise balanced photodetector optimized for the PT microscope was constructed to achieve shot noise-limited sensitivity at the indicated power level.

A balanced photodetector comprises two photodiodes and a transimpedance amplifier (TIA). Two Si-PIN photodiodes (Hamamatsu S1223-01) with photo-sensitive areas of 3.6 mm^2 and junction capacitances of 20 pF were used. For a TIA, the noise gain around the cutoff frequency increases with the junction capacitance of the photodiode, and the response speed of the circuit is reduced by the junction capacitance. As the junction capacitance increases with the photo activation area, an optimal photodiode that matched the diameter of the fiber bundle output port (3.4 mm) was selected. In the balanced photodetector, the difference in photocurrents between the two photodiodes was converted to voltage using the TIA. A 20-kΩ high load register was used to relatively reduce thermal noise in the circuit. The TIA was implemented using a high-speed FET operational amplifier (Linear Technologies LTC6268), and a phase compensating capacitor at 1 pF was connected in parallel with the load resister to reduce gain peaking. The photodetector had a −3 dB bandwidth of 7.8 MHz.

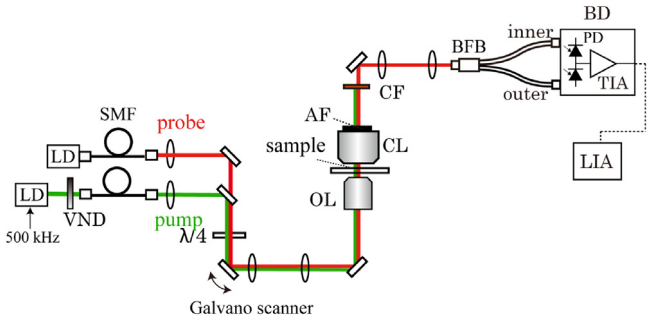

Fig. 1. Photothermal microscope setup with spatially segmented balanced detection using a bifurcated fiber bundle (BFB). LD: laser diode, SMF: single mode fiber, VND: variable neutral density filter, OL: objective lens, CL: condenser lens, AF: annular light block, CF: color filter, BD: balanced detector, LIA: lock-in amplifier.

The PT signals were demodulated using a broadband lock-in amplifier (NF LI5660), for which the time constant t_c was set to 10 μs. The optical system was the same as that in our previous publications except for the detector.

2.2. Experimental results and discussion

We first measured the noise level of the PT microscope at various probe power levels. The lock-in amplifier was used to measure the noise voltage density at a center frequency of 500 kHz. Fig. 2 shows that the noise voltage density in the absence of a probe beam (i.e., circuit noise) is $\delta v_c = 38\ \mathrm{nV}/\sqrt{\mathrm{Hz}}$. The noise equivalent power (NEP) was then calculated using the equation: $\delta v_c/\sigma G = 3.6\ \mathrm{pW}/\sqrt{\mathrm{Hz}}$. Here, $\sigma = 0.52$ A/W represents the photosensitivity of the Si-PIN photodiode at a wavelength of 780 nm, while $G = 2\times10^4$ V/A is the gain of the TIA. Circuit noise is mainly attributed to the thermal noise of the load register ($18\ \mathrm{nV}/\sqrt{\mathrm{Hz}}$) and voltage/current noise of the operational amplifier. The noise voltage density, δv, increases with P_{pr}; when P_{pr} exceeds ~0.02 mW, δv is mainly dominated by the shot noise, $\delta v_s = 2G\sqrt{q\sigma P_{\mathrm{pr}}}$, where q is the elementary charge. Note that δv is only 1.05-fold larger than δv_s when P_{pr} exceeds 0.1 mW.

In PT microscopy, the SNR is proportional to the square root of the probe power at the shot noise limit [17]. Therefore, a higher probe power is preferable for high-sensitivity imaging, assuming that the sample does not absorb the probe beam. In the following experiment, the LD output power for probing is set to the maximum ($P_{\mathrm{pr}} = 0.43$ mW). In this case, the probe power at the sample position was 3.5 mW. We confirmed about 6-folds improvement in the SNR compared with our previous system.

To evaluate the temperature increase and detection sensitivity of the shot noise-limited PT microscopy, we observed individual gold nanoparticles embedded in agarose gel. Gold nanoparticles absorb light at a wavelength near 520 nm as a consequence of surface plasmon