Phase-sensitive OCT imaging of multiple nanoparticle species using spectrally multiplexed single pulse photothermal excitation

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Abstract: We apply phase-sensitive optical coherence tomography to image multiple nanoparticle species with two excitation wavelengths matched to their distinct absorption peaks. Using different modulation frequencies, multiple species collocated within the sample can be distinguished. In addition, we characterize single-pulse excitation schemes as a method to minimize bulk heating of the sample. We demonstrate this new scheme with B-mode photothermal measurements of tissue phantoms.

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1. Introduction

Optical coherence tomography (OCT) is a well-established medical imaging system that is frequently used to investigate depth-resolved tissue structure at high speed and resolution [1]. Traditionally, OCT relies on the intrinsic scattering characteristics of a sample to create contrast [2]. Kuranov et al. were able to measure blood oxygen saturation level with dual-wavelength photothermal OCT using hemoglobin as the contrast agent [3,4]. In comparison, Robles et al imaged the absorption of hemoglobin directly using a novel spectroscopic OCT scheme while also using spectral features to distinguish oxy- and deoxy-hemoglobin [5,6]. However, exogenous contrast agents can provide unique sources of information and recently, there has been interest in advancing molecular contrast agents for OCT such as quantum dots [7], near-infrared dyes [8], and nanoparticles [9,10].

Here, we examine the use of nanoparticles as exogenous contrast agents in phase sensitive OCT. Some of the advantages of using nanoparticles include their high degree of biocompatibility and the absence of photobleaching. Nanoparticles also exhibit rapid thermal responses to optical excitation, leading to a readily detectable phase signature. The plasmon resonance of the nanoparticles is highly wavelength specific, which allows multiplexing multiple probes with distinct excitation spectra [11]. This direction has been examined previously. Adler et al. have demonstrated that phase-sensitive OCT can be used to detect photothermal absorption by gold nanoparticles [12]. Oldenberg et al. showed that by varying the size and shape of metallic nanoparticles, the resonant optical frequency can be precisely tuned [13]. Absorption at the plasmon resonance frequency causes highly localized temperature gradients around the nanoparticles, which in turn induces slight changes in the medium's refractive index [14]. These changes in refractive index can be detected using phase sensitive OCT with milliradian sensitivity.

Skala et al. have demonstrated molecular imaging with photothermal OCT by tagging the epidermal growth factor receptors (EGFR) in live cells with gold nanoparticles [15]. When cells containing both nanoparticles and high levels of EGFR were compared to cells with either low levels of EGFR or absence of nanoparticles, a three-fold increase in photothermal signal was observed [16]. Paranjape et al. have demonstrated detection of a single nanorose species in rabbit arteries [17]. In addition, Wang et al. have used nanoparticle species with dual-wavelength multifrequency photothermal wave imaging as an add-on modality to optical coherence tomography applied to detect macrophage and lipid in atherosclerotic plaques [18]. It has also been shown that nanoparticles can be used as scattering contrast agents with low backscattering albedo in highly scattering tissues such as breast tissues [16].

Despite the advantages of using nanoparticles as contrast agents, previous experiments have shown that bulk heating of the sample from repeated excitation can limit the signal to noise ratio (SNR) of the photothermal signal [12,15]. If care is not taken, the generated heat also has the potential to damage tissue structures as in targeted photothermal ablation. Therefore, there is a need to balance bulk heating effects with modulation and detection...
schemes that maximize SNR in order for nanoparticles to serve as effective contrast agents for in vivo photothermal based imaging.

In this work, we examine modulation techniques for photothermal OCT that can be used for simultaneous detection of several nanoparticle species. We demonstrate this technique by matching two excitation wavelengths at distinct temporal modulation frequencies with the plasmon resonances of different nanoparticle types to allow detection of both species at a single location. In addition, we characterize a single-pulse excitation scheme as a means to balance bulk heating of the sample with consideration of SNR. B-mode imaging of a tissue phantom with single-pulse excitation is presented in order to demonstrate the potential of using these methods for in vivo applications.

2. Methods

A fiber based spectral domain OCT (SD-OCT) system in Fig. 1(a) is used as the basis of our photothermal OCT system. Light from a superluminescent diode (SLD, $\lambda = 830$ nm, 50 nm bandwidth; Superlum, Russia) is focused through a 10× microscope objective (Zeiss) to a 25 $\mu$m-diameter spot on the sample. A galvanometer scans the beam across the back aperture of the objective, realizing a 1.2-mm field of view at the sample. A custom-built spectrometer with a spectral resolution of 0.049 nm and a maximum line rate of 53 kHz (Aviiva sensor model UM2) detects an interference signal generated by light from the sample mixed with that from the reference arm. A typical image of fingertip epithelial layers taken with this system is shown in Fig. 1(b).

To induce photothermal excitation of the nanoparticles, two diode pumped solid state lasers with excitation wavelengths of 532 nm and 405 nm are aligned collinearly with the OCT beam using dichroic mirrors before entering the galvanometer. Each laser is driven by a function generator that produces either square wave or single-pulse excitation. The maximum output power delivered to the sample by the 532 nm and 405 nm lasers were 22 mW and 17 mW, respectively. The lasers were chosen to match the peak absorption wavelengths of the nanospheres used in the following experiments, commercially obtained 60 nm diameter gold

![Fig. 1. (a) Schematic of the phase-sensitive photothermal SDOCT system (b) Typical OCT B-mode image of human epithelium (c) Measured absorption spectra of gold (60 nm diameter) and silver (40 nm diameter) nanospheres (d) Sample preparation](image)
and 40 nm diameter silver nanospheres (Ted Pella, Inc.) with absorption peaks at 530 nm and 410 nm, respectively. The absorption spectra of the two nanospheres as measured with a commercial spectrophotometer (Cary 300 Bio) are shown in Fig. 1(c).

In SD-OCT, a typical A-scan is computed by taking the magnitude of the Fourier transform of the spectral interference pattern. In order to measure the sub-coherence-length optical path delay due to the photothermal excitation, the phase angle of the depth-resolved complex signal is derived from the data. The photothermal signal is calculated by taking the difference in the phase measured above and below the depth of interest to determine the small-scale changes in path length caused by the temperature change within the volume.

In our experiment, the sample, an agar phantom containing both gold and silver nanoparticles, was sandwiched between two 100 µm coverslips spaced 140 µm apart with a spacer, as shown in Fig. 1(d). The concentration of the gold nanospheres was $2.3 \times 10^{10}$ particles/ml and the concentration of the silver nanospheres was $5.7 \times 10^{10}$ particles/ml. The photothermal signal was measured from the phase difference between the amplitude peaks of the two outermost air/glass interfaces of the sample, as indicated in Fig. 1(d). These points were chosen due to their strong reflectivity, which maximizes the phase sensitivity and hence the SNR of the photothermal signal.

3. Results

3.1 Phase stability measurement

To demonstrate the stability of the photothermal OCT system, the temporal phase drift from the agar sample was measured without photothermal excitation at a 35 kHz sampling rate. Figure 2(a) shows the measured phase signal at the top glass/air interface (top plot), the phase signal at the bottom glass/air interface (middle plot) and the difference of the two measurements (bottom plot). Note that the common phase noise present at both interfaces was effectively removed by this subtraction. In the following experiments, the phase difference of the two interfaces will be treated as the sample signal. In the absence of excitation of the nanoparticles, the phase stability of the sample was measured to be $\sigma = 1.54$ mrad, corresponding to an optical path length of 203 pm in water.

3.2 Multiplexing of two distinct nanoparticle species

The sample from Subsection 3.1 was simultaneously excited at 532 nm and 405 nm using two distinct modulation frequencies to demonstrate simultaneous detection of two distinct types of nanoparticles. The outputs of the two lasers (532 nm, 405 nm) were modulated with 1 kHz and 200 Hz square waves in order to excite the gold and silver nanoparticles, respectively. Figure 2(b) shows the detected time domain photothermal signal (inset) and the detected frequency domain signal. The thermal response of the sample to the external excitation appears as a low-pass filtered version of the square wave excitation and the overall time domain photothermal signal is a superposition of the two photothermal responses to the two input modulating signals. When the 30 ms time domain signal is transformed to the frequency domain, the two peaks corresponding to the excitation frequencies of the gold and silver nanoparticles are easily distinguishable. The SNR was calculated for both nanoparticles by taking the ratio of the peak amplitude at the modulation frequency to the standard deviation of 500 Hz range base noise around the modulation frequency without the photothermal excitation. The SNR of the photothermal signal was measured to be 473 and 322 for the gold and silver nanoparticles, respectively. The amplitude measurements in the Fourier domain for both gold and silver nanoparticles were performed across a range of concentrations, yielding linear graph shown in Fig. 3(a).
3.3 Bulk heating analysis with square wave modulation technique

One of the potential limitations for nanoparticle-based photothermal contrast in biological samples is the accumulation of bulk heating within the sample. Since optical excitation of nanoparticles near resonance creates highly localized temperature gradients within the medium, an imaged tissue sample is exposed to bulk heating that could be detrimental. To investigate bulk heating over the duration of excitation, the phase response of gold nanoparticles under 15 Hz excitation was measured for duration of 3.4 seconds (Fig. 2(c)). The periodic signal from the gold nanoparticles is superposed on an exponentially rising phase, characteristic of bulk heating. The total phase increase between the initial state and thermal equilibrium with modulation was 4.7 radians. For the given sample and the system setup, 4.7 radians in phase corresponds to 9.1°C in temperature, a potentially harmful increase. The conversion from phase to temperature was calculated using numerical method provided in Adler et al., for the sample at room temperature of 20°C [12]. The detected signal becomes more stable as the system reaches phase equilibrium, at which point the characteristic low-pass filtered square wave modulation dominates. The modulated phase at equilibrium is presented in high temporal resolution to show the stability of the modulated signal in Fig. 2(c) (inset) as measured with a sampling rate of 35 kHz and using 60 Hz excitation.
3.4 Single-pulse excitation technique

During the initial few excitation periods, the measured phase in Fig. 1(c) shows a large change for a given amount of energy absorbed. A single-pulse excitation technique utilizes this sharply rising region and therefore has a higher sensitivity than examining steady state periodic modulation at thermal equilibrium. As temperature is directly proportional to the overall phase change, this also minimizes bulk heating of the sample. The detected phase change by excitation of the gold nanoparticles with a single 400 μs pulse is shown in Fig. 2(d). Note the first order behavior in response to the square pulse, and the absence of bulk rise in phase over time. There is a sharp response present in the detected photothermal signal during the time of excitation, but immediately after the excitation pulse was shut off, the photothermal signal gradually degrades to the base level. The bulk phase increase was not present even when the 400 μs pulse was repetitively applied to the system in 1 ms interval as shown in Fig. 2(d) (inset). For the single-pulse excitation technique, the SNR of the detected phase was calculated by taking the ratio of the peak amplitude to the standard deviation of the noise in time domain. Using a 400 μs width pulse, a 140 mrad peak amplitude change was detected which resulted in an SNR of 59.4. A 20 mrad amplitude change was measured with a 20 μs width pulse excitation, which gave SNR of 9.7 as shown in Fig. 3(b).

3.5 B-Mode time scan of a tissue phantom

To simulate optical properties of a tissue, a phantom was prepared by adding TiO₂ powder, which increases the scattering coefficient of the sample to match that of tissue, to the agar solution containing gold nanoparticles with the identical geometry of the previous sample. The sample was divided into two regions: one region had only agar solution with TiO₂ and the other region was composed of both TiO₂ and gold nanoparticles in the agar solution as shown in Fig. 4(a). The concentration of TiO₂ was 0.3% (v/v) (scattering coefficient = 4.3 mm⁻¹) in both of the regions, and a gold nanoparticle concentration of 1.14 × 10¹⁰ particles/ml was used.

A B-mode image, consisting of the magnitude of the OCT signal from the tissue phantom is shown in Fig. 4(b). As shown in the image, the traditional B-mode amplitude scan does not provide contrast to distinguish the two regions with and without nanoparticles. When the region of interest was excited with a 1 ms width pulse from the 532 nm laser, single point phase measurements show a photothermal signal within the sample from the region with nanoparticles and none from the region without nanoparticles (Fig. 4(c)). For each single point phase measurement, 60 A-mode scans were collected in 3 ms. The B-mode magnitude image of the tissue phantom is false colored using the photothermal signal in Fig. 4(d), clearly...
separating the region with nanoparticles from the region without them. The sample was scanned point by point with photothermal excitation, and when the phase measurement at each point was compiled, the regions containing gold nanoparticles were revealed as shown in Fig. 4(e). The two arrows labeled as (i), representing a point scan of a region without nanoparticles, and (ii), representing a point scan of a region with nanoparticles, in Fig. 4(e) are shown as the single point measured photothermal signal in Fig. 4(c). Note the absence of photothermal signal in region (i) compared to region (ii). In the region (ii), there is an increase in photothermal signal during the 1 ms width single-pulse excitation period. When the excitation pulse ends, the photothermal signal gradually decreases as heat in the sample diffused into the nearby phantom medium. The thermal response for single pulses is presented in Figs. 4(c) and 4(e). The x-axis in the B mode time scan image corresponds to lateral dimension as in Fig. 4(d), and the y-axis in the image is the time dimension of the photothermal measurement as in Fig. 4(c). The total acquisition time of the B-mode time scan was 1 second.

Fig. 4. (a) Tissue phantom scheme showing a region containing nanoparticles and a region without nanoparticles (b) A typical OCT B-mode image of the tissue phantom (c) Measured phase response to the single-pulse excitation in the ROI containing gold nanoparticles and measured phase response to the single-pulse excitation in the ROI without gold nanoparticles (d) A typical OCT b-mode image of the tissue phantom false colored (e) B-mode time scan of the tissue phantom

4. Conclusion

Simultaneous use of both gold and silver nanospheres as contrast agents in photothermal OCT has been demonstrated with amplitude modulation and single pulse excitation techniques. Amplitude modulation detection techniques yielded high SNR while single-pulse excitation method reduced sample bulk heating with a reasonable SNR. Additionally, B-mode imaging of tissue phantoms with the single-pulse excitation scheme is presented in order to evaluate the potential of using these methods for imaging applications. Nanoparticles can be easily conjugated with antibodies and targeting moieties and therefore they can be used for applications such as targeting carcinoma cells, especially with their high biocompatibility [19]. In vivo application to tissue imaging would require additional considerations which balanced the optical properties of nanoparticles and tissue absorption. By increasing specificity with the multiplexing method and reducing bulk heating to minimize thermal
damage, the proposed scheme with photothermal optical coherence tomography potentially could widen the utility of the approach.

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