Molecular-specific imaging of tissue with photo-thermal optical coherence tomography

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Photothermal optical coherence tomography (PT-OCT) is a functional extension of conventional OCT with the potential to generate quantitative maps of molecular absorptions co-registered with the micron-resolution structural tomograms of OCT. Realizing this potential, however, requires refined understanding of the acquired signals and their dependence on system and sample parameters. Such understanding enables implementation of effective strategies for decoupling the various physical effects involved to obtain quantitative measures of molecular concentration/absorption. In this talk, we present an analytical model that considers the opto-thermo-mechanical properties of multi-layered samples in 3-D space, eliminating several assumptions that have been limiting previous models. The model is validated through experimental parametric studies, investigating the effect of sample and system parameters on acquired signals. The proposed model enables better understanding of the effects of system parameters and tissue opto-thermo-mechanical properties on experimental signals. Informed optimization of experimentation strategies is another outcome of proposed model, through which we designed and developed a spectroscopic transient-mode PT-OCT system for detection and differentiation of collagen and lipid at video rate.

Background – OCT is an interferometric optical imaging method capable of providing high-resolution (~10μm) cross-sectional images of tissue structures. The diagnostic value of OCT, however, is frequently limited by lack of chemical specificity. PT-OCT[1] is a pump-probe extension of OCT with the potential to complement the structural images of OCT with co-registered chemical/molecular information. To do so, the wavelength of the intensity-modulated photothermal laser (PT laser; i.e., pump) is selected at the peak absorption band of a molecule of interest (MOI; e.g., 1210nm for lipid). In this arrangement, absorption of the PT light by MOIs generates localized modulated thermoelastic expansions and thermo-optic variations that can be sensed by demodulation of the phase of a time-lapse OCT dataset (probe). Co-registered structural information is simultaneously obtained from the amplitude of the same OCT signal. PT-OCT's ability to produce chemically specific structural images is particularly valuable for applications where the structural signals alone provide insufficient diagnostic information (e.g., identification of rupture-prone atherosclerotic plaques). Quantitative PT-OCT imaging of tissue, however, is complicated by the fact that the PT-OCT signal is influenced not only by the concentration of MOIs but also the optical, thermal, and mechanical properties of tissue. Decoupling the effects of MOI light absorption from other influence parameters requires refined understanding of the complex physics underlying the PT-OCT signal. In this keynote talk, we will discuss our recent 3D opto-thermo-mechanical model for predicting PT-OCT responses in multilayer geometries. This improved model is key to understanding the origins of PT-OCT signals in tissue and the effects of system and sample parameters on experimental signals. We will also present and discuss our latest works focused on spectroscopic imaging of lipid and collagen at video rate with our recently introduced transient-mode PT-OCT (TM-PT-OCT) innovation[2].

Theoretical Model – The source of molecular-specific contrast in PT-OCT is the absorption of PT laser photons by MOIs. Such absorptions lead to formation of local thermal wave fields around the absorbers, and by extension, local modulated thermoelastic expansions. Due to the thermal expansions, the physical length of the sample will change, accompanied also by a variation of the temperature-dependent refractive index. These effects collectively cause a variation in optical path length (OPL) which can be sensed by a phase sensitive OCT system. That is, if the intensity of the PT laser is modulated in a sinusoidal form at a specific modulation frequency, the ensuing temperature field and OPL variations will also modulate at the same sinusoidal frequency. Accordingly, by applying Fourier transformation (FT) to the acquired time-lapse OCT phase signal (aka. M-scan) and evaluating the resulting spectrum at the PT-laser modulation frequency, the modulation amplitude $\Delta\phi$ can be measured at each depth. Based on this sequence of physical phenomena, we proposed a theoretical model to simulate the PT-OCT signals in samples in 3-D space using cylindrical coordinates. The model is comprised of three computing blocks in series: light field, thermal field, and stress/strain field. Each block is fed by the results of the previous block(s), system parameters, and material properties. In the first block, based on the optical properties of the sample and system parameters (e.g., PT laser power and modulation frequency), the PT laser irradiance in the sample is estimated in 3D. The temperature field in the sample is then determined in 3D considering the thermal properties and the light intensity distribution in the sample. The output of this part is the variation of temperature at every point in the sample over time. In the last block, the mechanical stress/strain field in the sample in response to the temperature change and as a function of the material's mechanical properties is obtained. Eventually, the OPL variation and PT-OCT signal are calculated from the mechanical displacements and the temperature changes.

Methodology– The experimental system used in this study[3], Fig. 1(a), is a dual-wavelength spectral-domain PT-OCT system containing a broadband superluminescent diode centered at 1310nm (+/- 75 nm at 10dB), a 2048-pixel line scan camera spectrometer with a maximum acquisition rate of 147 kHz, and two intensity-modulated PT laser at 1040 and 1210nm for detecting collagen and lipid, respectively. The axial and lateral resolutions of the OCT system are both 10µm. Performance test carried out on the system shows a relative displacement error of 3nm at a SNR of 35dB and sensitivity of >100dB which is close to that of a shot-noise-limited system. PT laser modulation pattern was in sinusoidal waveform shape for conventional PT-OCT experiments and in form of a short laser pulse (~400µs) for transient-mode PT-OCT experiments, Fig. 1(b).

For validating the theoretical model, PDMS phantoms containing different concentrations of absorbing dye (IR-806, Sigma Aldrich, USA) were prepared. Mayonnaise and chicken cartilage were used as lipid and collagen samples in spectroscopic TM-PT-OCT experiments.

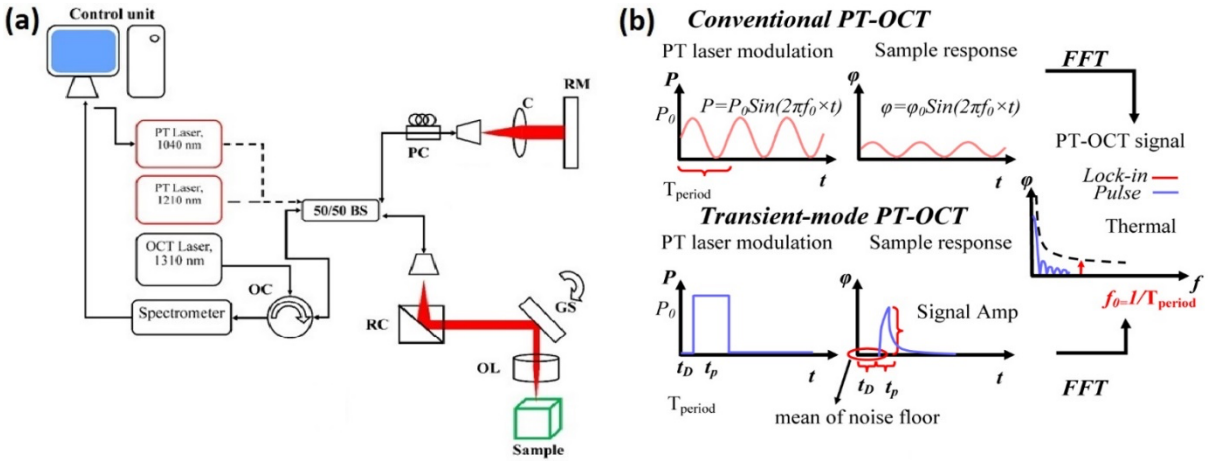

Fig. 1. Schematic of **(a)** experimental PT-OCT system; **(b)** laser modulation and sample responses in conventional PT-OCT and transient-mode PT-OCT.

Results and Discussion – Fig. 2(a) depicts simulated and experimental results of a sample with 2.2mg/ml of absorbing dye concentration (μ_a=10cm^{-1}) at various PT laser powers. Both simulated and experimental signals exhibit monotonic increase with depth. This trend is due to the cumulative nature of PT-OCT signal in a homogenous layer with the signal amplitude at each depth encoding the effect of all preceding layers. It can also be seen that PT-OCT signals exhibit a jump in amplitude at the sample surface (i.e., the Y-intercept) followed by an increase in signal values along depth. The rate of increase in signal values is higher at depths closer to surface because top surfaces of the sample are exposed to higher intensity of PT beam than deeper sample areas which results in larger thermo-elastic expansion of top levels. In Fig. 2(b), the average and standard deviation of experimental PT-OCT signals within a 20μm depth range below the sample surface are plotted in terms of PT laser power at various modulation frequencies. As seen at each modulation frequency, increasing the power on the sample increases the amplitude of the PT-OCT signal. These experimental observations are consistent with the predicted linear increase of the model. Deviations of experimental values from linearity are likely due to noise. Such deviations are larger at lower modulation frequencies due to the pink nature of noise in PT-OCT systems. Fig. 3(c) depicts the simulated and experimental responses of sample as a function of PT laser modulation frequency at 3 different laser powers. The inverse relation of PT-OCT signal strength with increase in laser modulation frequency is characteristic behavior of thermal wave fields. This trend, however, does not necessarily mean that lower modulation frequencies have priority to higher ones because in practice PT-OCT systems suffer from pink noise.

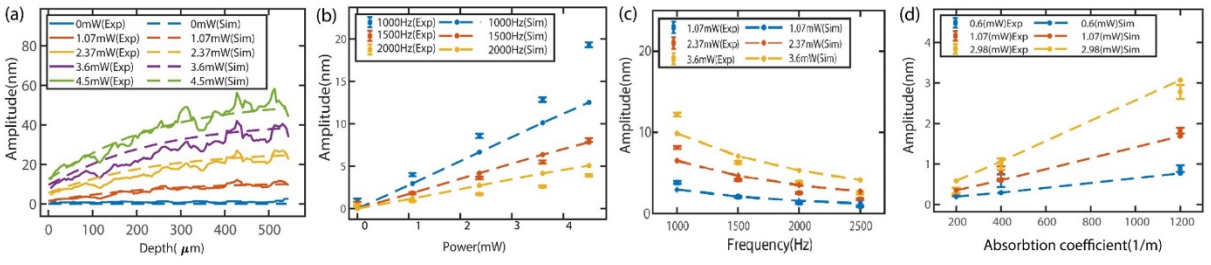

Fig. 2. (a) Effect of sample position with respect to system focal plane on PT-OCT signals. Simulated and experimental PT-OCT signals at **(b)** various PT laser power, **(c)** various PT laser frequencies, and **(d)** various absorption coefficients (MOI concentrations).

To understand the effect of the absorber concentration on PT-OCT signals, single layer samples with three different concentrations of absorber (0.22, 0.43, and 1.3mg/ml) with absorption coefficients (μ_a) of 100, 200, and 600 m^{-1} were imaged under PT illumination at various powers (0.6, 1.07, 2.98mW),

Fig. 3(d). As expected, there is a proportional relation between PT-OCT signal and dye concentration. The slopes of these lines are also directly correlated to the power of the PT laser. At higher concentrations, PT light is absorbed more efficiently, leading to the generation of more heat and eventually larger temperature variations. The greater temperature leads to a greater variation in OPL and subsequently greater PT-OCT signals. Therefore, the effects of the dye concentration and the PT laser power on the PT-OCT signals are identical and cannot be directly distinguished from each other as both parameters influence the thermal energy delivered to the sample. One possible way of decoupling the effects of MOI concentration from PT laser power is to perform spectroscopic PT-OCT at dual PT wavelengths. However, key downside to this approach is the dramatic decrease in imaging speed. To address this issue, we introduced transient-mode PT-OCT (TM-PT-OCT) which forms images based on transient thermal responses of MOIs to short PT laser excitations, Fig. 1(b). This approach enables PT-OCT imaging at high effective A-line rates of 1.5-7.5 kHz which is sufficient for video rate spectroscopic imaging of tissue.

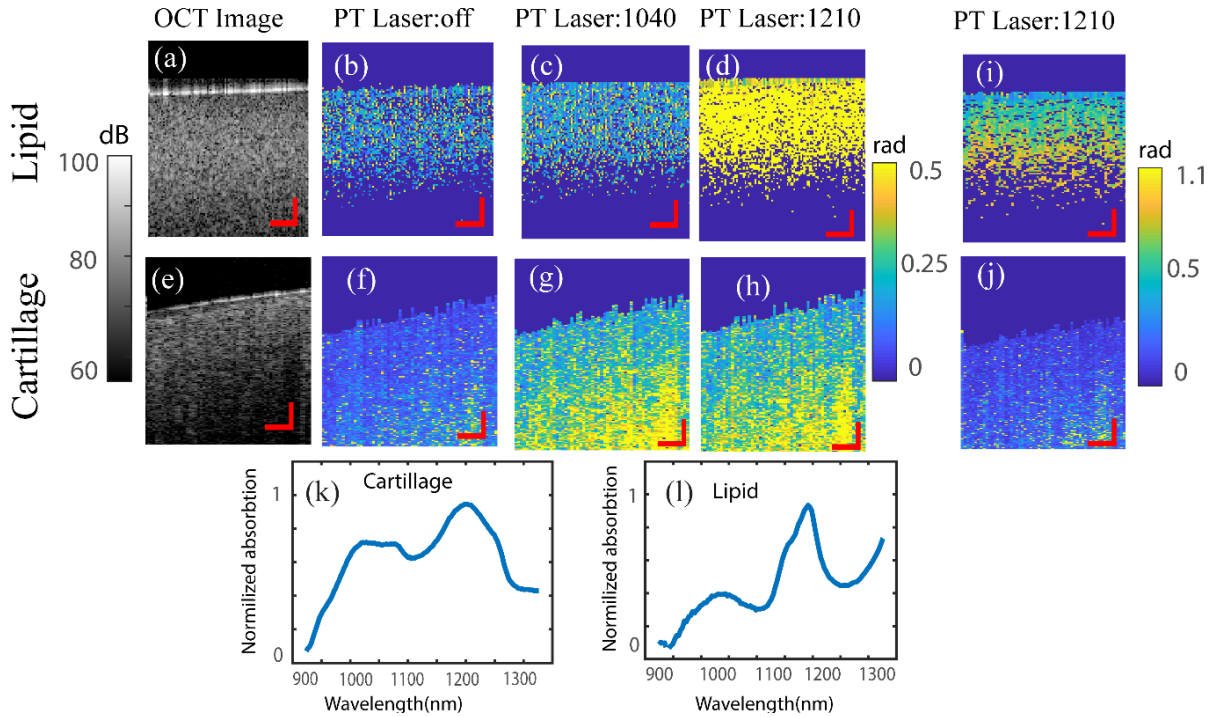

Fig. 3. **(a)** OCT image and **(b-d)** PT-OCT images of lipid, **and (e)** OCT image and **(f-h)** PT-OCT images of collagen samples, absorption spectrum of **(k)** lipid **(l)** collagen, and PT-OCT images of **(i)** lipid and **(j)** collagen with wider color bar.

Fig. 3 shows results of spectroscopic transient-mode PT-OCT for detection and differentiation of collagen and lipid. Panels (a) and (e) depict structural OCT images of lipid and cartilage, respectively, while panels (b) and (f) depict the baseline PT-OCT images of the samples when PT laser was off during imaging. Comparison of lipid 1040nm TM-PT-OCT image (Fig. 3c) with the baseline image (i.e., PT laser off; Fig. 3b), confirms absence of PT-OCT signals from lipid at 1040nm (as expected). At 1210nm (Fig 3d), on the other hand, strong TM-PT-OCT signals are registered from lipid. In the case of collagen, moderate TM-PT-OCT signals are observed for both 1040nm and 1210nm compared to the base-line image (Figs. 3g-h vs Fig. 3f). Results of Fig. 3 demonstrate the ability of TM-PT-OCT in registering the characteristic absorption spectral responses of lipid and collagen. Also, the spectrum obtained from a handheld spectrometer from the lipid and collagen samples (Fig 3.k and l) confirm this spectral response of the samples. TM-PT-OCT images at 1210nm are replotted with a wider colormap in Figs 3.i and 3.j to provide better appreciation of the difference between absorbance of lipid and collagen at

1210nm. As seen in Figs 3.i and 3.j, the signal from lipid is much stronger than those of collagen at 1210nm because of the greater absorbance of lipid at 1210nm.

References

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