Photoacoustic spectrum analysis for microstructure characterization in biological tissue: A feasibility study

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This study investigates the feasibility of characterizing the microstructures within a biological tissue by analyzing the frequency spectrum of the photoacoustic signal from the tissue. Hypotheses are derived from theoretical analyses on the relationships between the dimensions/concentrations of the photoacoustic sources within the region-of-interest and the linear model fitted to the power spectra of photoacoustic signals. The hypotheses are validated, following the procedures of ultrasound spectrum analysis, by simulations and experiments with phantoms fabricated by embedding the polyethylene microspheres in porcine gelatin, indicating that photoacoustic spectrum analysis could be a potential tool for characterizing microstructures in biological samples. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4768703>]

Photoacoustic (PA) imaging is a non-invasive modality that physically combines the high resolution of ultrasonography and the functional contrast of optical imaging. Although, in some cases, the macroscopic transitions of tissue optical properties in PA images can be evaluated through visual observation, the less significant contrast fluctuations within each seemingly homogeneous region is usually ignored. These small signal fluctuations, excluding the system noises, actually encode the dimensions and optical absorption contrasts of the microstructures within the imaged domain. In former research, the extraction and visualization of the microstructure information from ultrasound (US) signals has been extensively investigated using the methods of spectrum analysis. US spectrum analysis has shown promise in the detection and characterization of cancer^{1,2} as well as diseased tissues in liver³ and blood vessel.⁴ The principle of US spectrum analysis is to characterize the acoustic scattering properties of the microstructures within the region of interest (ROI) by observing several key factors, e.g., slope, intercept, and midband fit, of the linear models fitted to the truncated signal power spectra within a predetermined frequency interval.⁵ The utilization of Linear model is due to the fact that the spectra of US signals in dB usually monotonically increase or decrease following quasi-linear shapes.³ Former studies to validate the capability of US spectrum analysis in quantifying the dimensions and concentrations of acoustic back-scatterers within biological tissues indicated that the slopes of the linear models reflect the dimensions of the ultrasonic scatterers, and the intercepts encode both the dimensions and concentrations of the scatterers within the ROI.^{6–8}

PA signals, i.e., the US waves generated from light illumination of optically absorbing objects, demonstrate tissue contrast completely different from those revealed by US pulse-echo signals. Examination of PA power spectrum, employing the similar methods as in US spectrum analysis, may facilitate characterization of microscopic features in a

biological tissue based on the tissue optical properties. Pioneering phantom study⁹ by Yang *et al.* confirmed the feasibility of differentiating the dimensions of microspheres by PA power spectrum analysis. *In vivo* study with a prostate cancer murine model by Kumon *et al.* suggested the potential differentiating cancerous tumors from normal tissues based on the differences in PA spectrum parameters.¹⁰ In order to fully understand the mechanism and validate the feasibility of PASA for tissue characterization, this study investigated, by simulations and experiments with phantoms, the relationship between each spectrum parameter and the dimensions and the concentrations of optical absorbing sources.⁵ Unlike the study on biological tissues which usually involve complicated structures and inhomogeneous optical properties, the phantom study allows more precise control of the sample parameters and, therefore, more convincing comparison between simulation results and experimental findings.

The scope of this study is limited to the simplest case of analyzing the power spectra of one-dimensional PA signals, which are generated by spherical optical absorbers. The optical absorbers with various diameters and concentrations are uniformly distributed in optically transparent and acoustically homogeneous background materials. Such setup facilitates the analytical decomposition of the PA signals, in either frequency domain or time domain, to a series of convolution and dot products between the system factors, including the signal profile of a single spherical PA source, the spatial (or temporal) locations of the PA sources with respect to the ultrasonic transducer, the receiving directivity function of the transducer, and the acoustic attenuation of the medium as a function of frequency. A series of hypotheses will be deduced by the theory of signal processing and extensively validated by simulations and experiments.

The profile of a small spherical PA source in time domain can be derived from the PA wave equation,¹¹

$$p(\vec{r}, t) = \frac{1}{4\pi v_s} \frac{\partial}{\partial t} \left[\frac{1}{v_s t} \int d\vec{r}' p_0(\vec{r}') \delta \left(t - \frac{|\vec{r} - \vec{r}'|}{v_s} \right) \right], \quad (1)$$

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FIG. 1. An example of the PA signals and the signal power spectra in simulations and from a phantom containing optical absorbing microspheres with radius of $300\ \mu\text{m}$ and concentration of 120 per $1.5^3/\text{cm}^3$. (a) Simulated PA signal profile from a single microsphere. The magnitude is normalized to that generated by a $100\text{-}\mu\text{m}$ spherical PA source. The spikes in the insertion demonstrate the locations, on time axis, of the microspheres in the phantom. (b) Simulated signal sequence from the phantom. The insertion on the top shows the receiving directivity function of the transducer, and the insertion on the bottom shows the acoustic attenuation as a function of frequency. (c) The power spectrum of the signal in (b) and the corresponding linear fit in the frequency range of [0.5 MHz, 13 MHz]. (d) Signal profile from a single microsphere measured in experiment. (e) Signal sequence measured in experiment. (f) Power spectrum of the signal from the phantom calibrated by the frequency response of the hydrophone (shown in the insertion) and the corresponding linear fit.

where $\vec{r}$ and $\vec{r}'$ are the positions of the transducer and PA source, respectively; v_s is the speed of sound in the specific medium; p_0 is the initial pressure magnitude at the PA source. Evaluation of Eq. (1) on a spherical PA source with the radius of r generates a bipolar signal profile with temporal width of $2r/v_s$,¹¹ as annotated in the signal profile generated by a $300\text{-}\mu\text{m}$ radius spherical PA source shown in Fig. 1(a). Nonetheless, the microspheres used in the phantom experiment of this study also produce noticeable signal ringdown, as shown in Fig. 1(d). The ringdown can be simulated as a continuous series of single signal profiles modulated by an exponentially decaying contour, as shown in Fig. 1(a). The power spectrum of the decaying contour apparently contributes to the low frequency components of the entire signal power spectrum.

The PA signals from multiple PA sources could be mathematically expressed as the single PA source profile convoluted by a spike function sequence with the spatial or equivalent temporal locations of the PA sources. Such convolution is equivalent to the spatial integration of the signals generated by all the PA sources. In this study, we assume that the spatial distribution of PA sources in biological follows a uniform random distribution. The autocorrelation of a uniform random distribution function $u(t_1, t_2)$ with element magnitudes of $\{0, 1\}$ can be estimated by a triangle function,

$$R_{uu}(\tau) = \begin{cases} \frac{N}{t_2 - t_1} \tau + N & t_1 - t_2 < \tau < 0 \\ \frac{-N}{t_2 - t_1} \tau + N & 0 < \tau < t_2 - t_1 \end{cases}, \quad (2)$$

where N is the number of non-zero components within the evaluated interval, or the number of spikes within the signal sequence. The square of Fourier transform of Eq. (2), i.e., the power spectrum, apparently has large low frequency components. The magnitude of the power spectrum of Eq. (2) increases with the increase of N . However, we do not expect significant variations of the linear model fitted to the power spectra with respect to the changes of N value, because most of the very low frequency components will be truncated before the linear regression.

The signal amplitude for each PA source is affected by the transducer receiving directivity function and the acoustic attenuation as a function of the frequency. The receiving directivity function of the transducer can be quantified as

$$\alpha_{\text{directivity}}(\theta) = \text{Re}\{J_{\text{inc}}[\pi \cdot D\lambda^{-1} \cdot \sin(\theta)]\} \\ = \text{Re}\left[\frac{J_1(\pi \cdot D\lambda^{-1} \cdot \sin(\theta))}{\pi \cdot D\lambda^{-1} \cdot \sin(\theta)}\right], \quad (3)$$

where D , the diameter of the transducer, is 1.5 mm for the needle hydrophone used in this study; λ , the wavelength of the PA signal to be received, is approximately 0.15 mm with the speed of sound of 1500 m/s and the central frequency of the hydrophone of 10 MHz; and θ is the incident angle of the PA wave with respect to the receiving transducer. The directivity function within the angular range of $[-\pi, \pi]$ is plotted in the top insertion in Fig. 1(b). The attenuation of PA signals increases linearly with respect to the square of the frequency.¹² From our preliminary experiments, we estimate the (α/v^2) ratio of 8% porcine gel phantom at 20 °C as 750

which agrees with the findings in Ref. 12. The frequency-related acoustic attenuation in our gel phantom is plotted in the bottom insertion in Fig. 1(b). Due to the high frequency resistant characteristics, the acoustic attenuation reduces the slope of the linear fit to the power spectrum.

The signal generated considering all the factors mentioned above is shown in Fig. 1(b). The corresponding power spectrum in dB is shown in Fig. 1(c). An approximately 30 dB range of the spectrum [0.5 MHz, 13 MHz] are fitted to a linear model with least-square method. Three parameters of the linear model are extracted: (1) slope; (2) intercept on the y axis; and (3) midbandfit, which is the magnitude of the linear model at the center of the observed frequency interval, as is marked in Fig. 1(c).

A series of hypotheses regarding the relationship between the dimensions and concentrations of the PA sources and the parameters of the linear fit to the power spectra can be summarized according to the analysis of the PA signals:

- (1) The slope decreases as the PA source dimension increases, because the signal center frequency shifts toward low frequency as a result of the expansion of the duration of the bipolar signal profile;
- (2) The slope generally holds as the PA source concentration varies, because most of the very low frequency component introduced by Eq. (2) is excluded before the linear fitting process. However, we expect the slope slightly decreases when the concentration increases significantly. This is due to the fact that the frequency components introduced by Eq. (2) extend into the high frequency range and thereby cannot be completely excluded from the linear fitting. The contribution of the spectrum residuals of Eq. (2), although ignorable when N value is relatively small, could become observable when N value is significantly increased.
- (3) The intercept and the midband fit increase as the PA source dimension increases, because the volume determines the total optical absorbance and, consequently, the magnitude of the pressure generated from the PA source.
- (4) The intercept and the midband fit increase as the PA source concentration increases, as the total optical absorbance within the ROI rises.

Each of the hypotheses is examined by simulations and experiments in the following sections.

The simulation was conducted on 25 samples containing 5 different microspheres sizes (diameters of 100, 200, 300, 400, and 500 μm) at 5 different concentrations (30, 60, 120, 240, 480, microspheres per 1.5^3 cm^3). In each sample, the microspheres, i.e., the PA sources, were distributed uniformly and randomly in a 1.5^3 cm^3 cubic volume. The transducer was 30 mm away from the sample, with the receiving direction aligned to the center of the cubic sample. The spatial separation between the microspheres and the transducer were converted into a sequence of temporal spike functions, as shown in the insertion in Fig. 1(a). As mentioned above, the convolution of the signal profile from a single spherical PA source with the spike function produces the PA signal sequence, as shown in Fig. 1(b). The acoustic attenuation was introduced by a $2\text{-}\mu\text{s}$ sliding window with frequency attenuation per unit distance multiplied by the center distance between the slide

window and the transducer. The calculated signal power spectrum, utilizing the Welch's approach¹³ with a $2\text{-}\mu\text{s}$ hamming slide window with 30% overlapping of each step, is shown in Fig. 1(c).

Resembling all the parameters in simulations, the phantoms for experiments were fabricated with polyethylene microspheres (Fisher scientific LLC.) contained in porcine gels made from water solution of 8% porcine gelatin powder (Sigma-Aldrich Co. LLC.). The polyethylene microspheres are black in color, with strong optical absorption in the visible spectrum range. After being heated thoroughly, the gelatin-microsphere solution was poured into a cylindrical mold with 25.4 mm in diameter and 60 mm in depth. In total, 25 phantoms were fabricated. The diameters and concentrations of the microspheres in each phantom were identical to those in the simulations. Another blank gel phantom was also made, for the detection of background PA signal for calibration.

As shown in the experiment setup in Fig. 2, the optical illumination was provided by a Q-switched Nd-YAG laser (PrecisionLite DLS 8010, Continuum) with wavelength at 532 nm, output power of 800 mJ per pulse, and pulse duration of 5 ns. A pair of concave and convex lenses expanded the light beam to approximately 15 mm in diameter, and then a 50-50 beamsplitter bisected the light beam for the bilateral illumination of the phantom submerged in a water tank. A needle hydrophone (HNC-1500, ONDA Co.) with detection bandwidth of 20 MHz was positioned perpendicular to the top surface of the phantom at a distance of 1 mm from the surface. The light beams were projected to an area in the phantom that was 30 mm below the top surface, avoiding direct light illumination on the hydrophone which could induce strong noise. A digital oscilloscope (TDS 540, Tektronix, INC.), which was synchronized to the laser firing, acquired the signals from the hydrophone at a sampling rate of 250 MHz. The axis of the light beam was aligned to those of the hydrophone and the cylindrical phantom. One typical PA signal sequence is shown in Fig. 1(e). Treated in the same way as in simulation, the signal spectra were calculated with Welch's approach,¹³ and then calibrated with the frequency response of the hydrophone as shown in the inset in Fig. 1(f). In the power spectrum in Fig. 1(f) from the

FIG. 2. Schematic of the experimental setup. The signal acquisition by oscilloscope is synchronized to the laser firing with Q-switch trigger. The light beam is split and bilaterally illuminates the phantom.

FIG. 3. Plots of linear regression parameters, including the slope, intercept and midband fit, with respect to the diameters and concentrations of the micro-spheres in the phantom. Left column: simulation results. Right column: experimental results.

experiment, the signal-to-noise ratio is close to 1 when the frequency is above 13 MHz. At the extremely low frequency part below 0.5 MHz, we noticed very strong spectrum amplitude which was not from the light illuminated microspheres but instead from the residual background signal produced by the laser illumination on the gel. Therefore, in this study, the linear regression in both simulations and experiments were restricted to a frequency interval of 0.5–13 MHz. As can be seen in Figs. 1(c) and 1(f), the dynamic range of the power spectrum in this usable frequency interval is approximately 30 dB.

The linear regression parameters, including the slope, intercept and midband fit, of the PA signals acquired from simulations and experiments as functions of the diameters and the concentrations of the PA sources in the phantoms are

shown in Fig. 3. The simulation results are arranged in the left column and the corresponding experimental results in the right column. To present the mean and the standard deviation values, each simulation was generated statistically from 30 independent trials with randomly generated PA source locations. For the experiment on each phantom, 6 independent measurements were made at different locations on the top surface of the phantom. Good agreement between the simulations and experiments has been achieved. Especially in the top lines in Fig. 3 demonstrating the slope as functions of the diameters and the concentrations of the PA sources, most simulation results can quantitatively match the experimental findings. Figs. 3(c)–3(f) present the intercept and the midband fit of the linear regression as functions of the diameters and the concentrations of the PA sources in the

phantom. For each study, the simulation and the experiment show not only the same trend but also the almost identical dynamic ranges of the values. The differences in the absolute values from the simulations and the experiments are due to the lack of the knowledge regarding the exact pressure values at the origins of the PA sources. Close observation of the Fig. 3(c) reveals that, in agreement to hypothesis 2, the slope slightly decreases with respect to the increase of PA source concentration. However, the decreasing is very slow and in the experiments, the trend is almost overwhelmed by the fluctuation due to the system noise, as shown in Fig. 3(d).

Note that the three parameters extract from the linear models are not independent. The quantification of any two of the three can be used to derive the third. For instance in Fig. 3, despite the seemingly correlation between the intercept and midbandfit, the knowledge of any two points on a linear model can be used to determine the linear model and consequently the slope value. The parameter of midbandfit is thereby independent from the intercept. According to our previous study,¹⁰ midbandfit could better represent of the energy level of a power spectrum because it describes the averaged elevation of the linear model. This study focuses on phantom study in which the background signal can be simply removed with a black phantom without PA sources. For actual measurement in biological tissue, the background signal can be removed by band pass filter, as the inset of Fig. 2(c) indicated that the background signal majorly consists of a large low frequency component and high frequency fluctuations. This study only investigated the randomly distributed microspheres with identical sizes and homogenous concentrations. Yet in real biological tissue, the microstructure dimensions and densities could vary within a region of interest. Nonetheless, we expect that various tissue types can still be discernible by statistical distributions of the dimensions and densities. Therefore, our conclusion on the relationship between the microstructure dimensions as well as densities and the spectrum parameters should still hold. The PA spectra are only qualitatively compared in this study. The quantification of microstructures with PA spectrum analysis requires, in addition, the accurate modeling of laser energy delivered to the PA sources, which will be further investigated in our future works.

The feasibility of PASA for characterization of the microstructures in a biological sample is investigated through theoretical analyses, simulations, and phantom experiments. The classic method for US spectrum analysis has been employed for frequency-domain analysis of PA signals. A series of correlations between the PA sources, including dimensions and concentrations, and the parameters of the linear fit to the PA power spectra have been established, suggesting that PASA could potentially decode the microscopic

information included in PA images and lead to more quantitative and objective characterization of the biological tissue under investigation. Current study is confined to one-dimensional A-line signals and phantoms with simple geometries. However, all the conclusions can potentially be extended to two- or three-dimensional images and biological samples containing optical absorbers with various dimensions, shapes, and concentrations, as long as the system instrumentation possesses sufficient bandwidth and signal acquisition rate.

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