Quantitative photoacoustic measurement of tissue optical absorption spectrum aided by an optical contrast agent

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Abstract
In photoacoustic imaging, the intensity of photoacoustic signal induced by optical absorption in biological tissue is proportional to light energy deposition, which is the product of the absorption coefficient and the local light fluence. Because tissue optical properties are highly dependent on the wavelength, the spectrum of the local light fluence at a target tissue beneath the sample surface is different than the spectrum of the incident light fluence. Therefore, quantifying the tissue optical absorption spectrum by using a photoacoustic technique is not feasible without the knowledge of the local light fluence. In this work, a highly accurate photoacoustic measurement of the subsurface tissue optical absorption spectrum has been realized for the first time by introducing an extrinsic optical contrast agent with known optical properties. From the photoacoustic measurements with and without the contrast agent, a quantified measurement of the chromophore absorption spectrum can be achieved in a strongly scattering medium. Experiments on micro-flow vessels containing fresh canine blood buried in phantoms and chicken breast tissues were carried out in a wavelength range from 680 to 950 nm. Spectroscopic photoacoustic measurements of both oxygenated and deoxygenated blood specimens presented an improved match with the reference when employing this technique.

1. Introduction

Photoacoustic tomography (PAT), also referred to as thermoacoustic or optoacoustic tomography, is an emerging biomedical imaging technique which has drawn considerable interest in the past decade [1-9]. In PAT, a short-pulsed laser source is used to illuminate a biological sample. The laser-generated photoacoustic signals that are excited by thermoelastic expansion resulting from a transient temperature rise on the order of 10 mK can be measured by a wide-band ultrasonic transducer. The acquired signals can then be used to rebuild the distribution of optical energy deposition within the sample. The amplitude and the time of arrival of the photoacoustic (PA) signal provide respectively the information about the intensity and the spatial distribution of tissue optical absorption [10,11]. PAT images have the advantages of the inherent high contrast of diffuse optical imaging and the high spatial resolution of ultrasonography.

It is known that all chromophores (e.g. water, oxy-hemoglobin, deoxy-hemoglobin, lipid, cytochrome oxidase and melanin) in biological tissues have characteristic spectroscopic optical absorption features. These are essentially the fingerprints which allow these chromophores to be uniquely identified. Various tissues, containing different concentrations of chromophores, also show different optical absorption spectra. The spectroscopic form of PAT (i.e. SPAT), which employs multiple wavelengths to image a sample, provides a potentially new method.
for quantifying various chromophores and monitoring physiological properties in the target tissue with high sensitivity and high spatial resolution. In other words, SPAT enables not only morphological imaging based on tissue optical contrast but also quantitative evaluation of molecular conformation of biological tissues. As an example, based on the differences between the absorption spectra of oxygenated hemoglobin (HbO\textsubscript{2}) and deoxygenated hemoglobin (Hb), imaging of blood oxygen saturation and blood volume has been explored using the SPAT technique [5-8,12,13]. Moreover, SPAT also shows prominent sensitivity to a variety of optical contrast agents, including both organic dyes and metallic nanocolloids, and holds promise in revealing physiological and biochemical information of living tissues by imaging exogenous bioactive contrast agents [14,15].

The PA signal induced by light pulses is proportional to the optical energy deposition at the target tissue, which is the product of the tissue optical absorption coefficient and the local light fluence. The optical properties including the absorption coefficient, scattering coefficient, refractive index, and anisotropy factor of the target and the background tissues, are all highly dependent on the wavelength. Thus the spectrum of the local light fluence at the target tissue is different from the spectrum of the incident light on the sample surface. Therefore, the knowledge of the spectrum as well as the spatial distribution of light fluence in the sample is essential to achieve quantitative spectroscopic measurement or imaging with high accuracy [16,17]. Previous SPAT experiments either did not take into account the local light fluence in image reconstruction procedures [7,18] or included a correction factor to compensate the wavelength-dependent light attenuation from the excised tissue of the same type [5]. Mathematical model has also been used to extract the chromophore distribution from the measured PA signals [16]. However, due to the high complexity and heterogeneity of biological tissue structure, composition, optical properties and physiological parameters (e.g. oxygen saturation) that all affect the distribution and the spectrum of light fluence in the sample, all of the above methods have limited accuracy and cannot provide satisfactory compensation for quantitative photoacoustic measurement of subsurface tissues.

In this paper, we present a new technique that can directly measure the spectrum of local light fluence at a target tissue in a highly scattering medium by introducing an extrinsic optical contrast agent with known optical absorption spectrum. By using the measured PA spectra of the target tissue with and without the contrast agent, the spectrum of local light fluence could be determined with high accuracy. With the spectroscopic local light fluence determined, quantitative photoacoustic measurement of the optical absorption spectrum in the target tissue can be achieved without being affected by the morphological features and physiological parameters in the imaged sample. To examine the performance of this method, experiments on oxygenated and deoxygenated fresh canine blood specimens in vessels buried in optical scattering phantoms and chicken breast tissues were conducted, and SPAT outcomes were validated with the reference of the intrinsic optical absorption spectra of the measured blood specimens.

2. Methods

In whole human or canine blood, the two forms of hemoglobin (i.e. HbO\textsubscript{2} and Hb) are the dominant chromophores in the visible and near-infrared (NIR) spectral region. If \( \varepsilon \) is the molar extinction coefficient of the whole blood and \( \varepsilon_{\text{HbO}_2} \) and \( \varepsilon_{\text{Hb}} \) are those corresponding to HbO\textsubscript{2} and Hb respectively, the spectroscopic photoacoustic signal intensity from a blood specimen can be written as

\[
P_1(\lambda) = K \varepsilon(\lambda) C_\gamma \Phi(\lambda)
\]  

(1)
Where
\[
\varepsilon(\lambda) = \varepsilon_{\text{HbO}_2}(\lambda) \text{SO}_2 + \varepsilon_{\text{Hb}}(\lambda)(1 - \text{SO}_2).
\]

\(C_T = C_{\text{HbO}_2} + C_{\text{Hb}}\) is the total molar concentration of hemoglobin. \(\text{SO}_2 = C_{\text{HbO}_2}/C_T\) is the oxygen saturation of hemoglobin, \(\phi\) is the spectroscopic light fluence at the target blood specimen, and \(K\) is a constant that is independent of laser wavelength. The values of \(\varepsilon_{\text{HbO}_2}\) and \(\varepsilon_{\text{Hb}}\) in a wide wavelength range from the visible to the NIR regions are available in the literature \[19\]. As indicated in Eq. (1), \(\text{SO}_2\) of a blood specimen can be estimated if the values of \(P_1\) and \(\phi\) are known at two or more different wavelengths.

It is not difficult to measure the spectrum of the incident light fluence on the sample surface. However, because the optical properties of biological tissues are wavelength dependent, the spectrum of the local light fluence \(\phi\) at the target blood specimen will not be the same as the spectrum of the incident light. We have developed a new strategy of photoacoustic spectroscopy that can obtain the local light spectrum by introducing an extrinsic contrast agent that is an IR dye in this study (FHI 90011S, Fabricolor Holding Inc, New Jersey). If \(\varepsilon_{\text{dye}}\) is the molar extinction coefficient of the dye that is introduced to the blood sample, then the spectroscopic PA signal from the mixture can be written as
\[
P_2(\lambda) = K\varepsilon(\lambda) C_T \phi(\lambda) + K\varepsilon_{\text{dye}} C_{\text{dye}} (\lambda) \phi(\lambda),
\]
where \(C_{\text{dye}}\) is the molar concentration of the dye.

From Eqs. (1) and (3) we can obtain the local light spectrum at the target tissue by
\[
\phi(\lambda) = \frac{P_2(\lambda) - P_1(\lambda)}{K\varepsilon_{\text{dye}} C_{\text{dye}}(\lambda)}.
\]

Substitution of Eq. (4) in Eq. (1) yields the absorption coefficient of the blood sample that is given by
\[
\varepsilon(\lambda) = \frac{P_1(\lambda) \varepsilon_{\text{dye}} (\lambda) C_{\text{dye}}}{C_T [P_2(\lambda) - P_1(\lambda)]}.
\]

This equation gives the absorption spectrum of the blood specimen localized inside a biological sample that is free from the optical properties such as the scattering and absorption in the background tissue. This method is based on an assumption that the introduction of the optical contrast agent will not affect the spectroscopic local light fluence. This assumption should hold when the concentration of the introduced contrast agent is low and the size of the imaged object is small in comparison with the penetration depth of the light in the imaged object. Another assumption is that the chromophores in the target tissue such as hemoglobin will not have any chemical or physical reactions to the introduced contrast agent, because these reactions may change the intrinsic optical absorption spectra of these chromophores as well as the contrast agent.

3. Experimental procedure

The schematic of the photoacoustic setup is shown in Fig. 1. An optical parametric oscillator (OPO) system (Vibrant B, Optotek) pumped by an Nd:YAG laser (Brilliant B, Bigsky) was

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used as the laser source. The laser generated 5.5 ns duration pulses at 10 Hz pulse repetition rate with wavelength tunable from 680 to 950 nm. A laser beam along the Z-axis illuminated the sample with an incident energy density less than the ANSI safety limit. Micro-flow vessels were created by injecting fresh canine blood into a soft polyethylene (PE) tube (Intramedic, Clay Adams Brand) which is optically transparent and has an inner diameter of 0.58 mm. The vessel was aligned along the Y-axis with the center part exposed to the laser beam. Before reaching the sample surface, a small portion of the laser beam was extracted by putting a glass plate just before the sample and the reflected light was measured with a photodiode to monitor the incident light energy and the laser fluctuation. Neutral density filters (NDF) were used to reduce the power of the laser reaching the photodiode. A focused wideband transducer (V312, Panametrics) with a focal length of 19 mm, diameter of xx mm, -6 dB bandwidth of 133 %, and a center frequency of 10 MHz was used to collect the PA signals propagating along the X-axis. Both the sample and the transducer were immersed in water for acoustic coupling. The PA signals received by the transducer were amplified (PR5072, Panametrics) and fed into a channel of a 500-MHz digital oscilloscope (TDS 540B, Tektronics). Output of the photodiode was fed into another channel of the oscilloscope. The oscilloscope was triggered by the laser pulse and a computer was used to collect and store the data from the oscilloscope. Photoacoustic measurements were carried out in a spectral range from 680 to 950 nm with a constant interval of 5 nm for a total of 55 samples). At each wavelength, PA signals generated by 20 laser pulses were averaged to achieve a better signal-to-noise ratio. A complete set of spectroscopic photoacoustic measurements at the 55 wavelengths took about 40 min using the current system.

Fresh whole blood collected from dogs was used. Oxygenated blood was taken from the left atrium of the heart when the dog was alive, while the deoxygenated blood was collected from the venous-caudal vena cava, 5-10 min after the dog was sacrificed. A blood gas analyzer (ABL 5, Radiometer Copenhagen) was used to measure the oxygen level of each blood specimen. The infrared dye used as the contrast agent has strong absorption over the entire wavelength range from 680 to 950 nm (see Fig. 2). To keep the hemoglobin concentration constant before and after introducing the dye, only a small amount of highly concentrated dye solution (5 μL in 1 mL of blood) was added to the blood. This amount led to the concentration of the dye in the blood of 2.2 mM/L. With the measured PA spectra from the same blood specimen with and without the dye, the local light fluence was obtained by using Eq. (4). The PA spectrum of the blood sample without the dye was then divided by the local light spectrum to obtain the corrected spectrum of optical absorption of the blood specimen, as indicated by Eq. (5).

4. Results and discussion

A typical PA signal from a blood vessel received by our photoacoustic system is given in Fig. 3 along with the received photodiode signal of the laser pulse. The peak-to-peak amplitude of the bipolar PA signal is proportional to the local light energy deposition in the vessel. However, the total shaded area under the curve, which is indicated in Fig. 3 and was used in this study, can manifest the local light energy deposition with an improved signal-to-noise ratio. For the same reason, the shaded area marked on the photodiode signal was used to indicate the incident light energy on the sample surface. The ratio between the PA signal and the photodiode signal gave the photoacoustic measurement at each wavelength.

To validate the sensitivity and accuracy of our SPAT system in describing the optical absorption spectrum of a tissue, spectroscopic measurement of blood specimens was first conducted in a non-scattering medium. The fresh canine blood contained in the vessel was kept at a depth of 1 cm beneath the water surface during the measurement at all the 55 wavelengths. The oxygen saturation level (SO_2) for each blood specimen was measured with the blood gas analyzer. With the SO_2 of each blood specimen measured, the reference spectroscopic absorption of the blood specimen can be determined with the known molar extinction
coefficients of the oxygenated and the deoxygenated hemoglobin [19]. This reference, shown by the solid curve in each of the following figures, enables the verification of SPAT measurement. Figure 4 shows the spectroscopic photoacoustic measurements of an oxygenated (SO$_2$ = 1.00) and a deoxygenated (SO$_2$ = 0.33) blood specimens. Because this experiment was conducted in a non-scattering medium, the incident light fluence on the sample surface (i.e. the surface of the water) was very close to that at the target blood vessel, and hence the SPAT results can present the blood absorption spectra well without need for any correction. The PA outcomes match with the reference with goodness of fit R$^2$ of 0.999 for the oxygenated blood and 0.998 for the deoxygenated blood. R$^2$ is the sum of the squares of the relative errors. The good match between the SPAT outcomes and the reference has proven the high sensitivity and accuracy of our SPAT system.

To mimic the situation in a highly scattering medium such as a biological tissue, we made a phantom using whole milk diluted with water in the ratio of 1:4 [20-22]. Photoacoustic spectra of an oxygenated (SO$_2$ = 0.97) and a deoxygenated (SO$_2$ = 0.10) canine blood specimens immersed 7.5-mm deep in the diluted milk are given in Fig. 5 in comparison with the reference. Fig. 5(a) shows the PA spectrum of the oxygenated blood specimen without the compensation for the local light spectrum; while Fig. 5(b) is the PA spectrum of the same blood specimen after the compensation for the local light spectrum through the method described above. The spectrum of the local light fluence was obtained by using Eq. (5) with the PA spectra taken before and after introducing the contrast agent. By comparing Fig. 5(a) and (b), we can see that the SPAT spectrum after the compensation shows a better match with the reference. The goodness of fit R$^2$ improved from 0.978 to 0.988 for the oxygenated blood specimen. Figs. 5 (c) and (d) show the result for deoxygenated blood specimen. Again, after the compensation by considering the local light spectrum, the SPAT spectrum shows a better match with the reference. The goodness of fit R$^2$ improved from 0.963 to 0.993 for the deoxygenated blood specimen.

To further validate this technique, we also conducted the PA measurements of the blood specimens in vessels buried 3-mm deep in fresh chicken breast tissues. The PA spectra of an oxygenated (SO$_2$ = 0.98) and a deoxygenated (SO$_2$ = 0.27) blood specimens in a slab of chicken breast in comparison with the reference are shown in Fig. 6. For each blood specimen, we can see a clearly improved matching between the SPAT outcome and the reference after the compensation by introducing the optical contrast agent. By using the method described above, the goodness of fit R$^2$ was improved from 0.983 to 0.997 for the oxygenated blood specimen, and from 0.980 to 0.995 for the deoxygenated blood specimen.

Results of our photoacoustic experiments on oxygenated and deoxygenated blood specimens in a scattering medium such as the milk or the chicken breast tissue reveal that 1) the optical properties such as the scattering and absorption of the scattering medium affect the spectrum of the light fluence reaching the target tissue in the medium, which may result in a mismatch between the SPAT measurement and the true absorption spectrum of the target tissue; 2) the spectrum of the local light fluence at the target tissue such as a blood vessel within a scattering medium could be measured by introducing an optical contrast agent and then performing another SPAT measurement of the target tissue after the first SPAT measurement; and 3) the measured spectrum of the local light fluence could be used to compensate the SPAT result for better revealing of the optical absorption spectrum of the target tissue.

5. Conclusions

This work presents a novel method to measure the spectroscopic tissue optical absorption in a highly scattering medium by using the emerging SPAT technology aided by extrinsic optical contrast agents. Preliminary studies on phantoms and ex vivo chicken breast tissues have proven
the feasibility of this method. After the compensation by considering the local light fluence, a quantitative photoacoustic measurement of the subsurface tissue optical absorption spectrum can be achieved with improved accuracy. This technique makes SPAT a potentially powerful tool in mapping and quantifying chromophore distribution in biological tissues, which may contribute significantly to imaging and diagnosis at the molecular and genetic levels. Moreover, by using this method, highly accurate quantification of blood oxygen saturation in subsurface tissues can be achieved in a noninvasive manner without being affected by background tissues and sample surface. This may prove very useful in cancer diagnosis and characterization by grading objectively the hypoxia in a target tumor. In our future work, studies on living animals will be carried out to test the performance of this technique in vivo. Moreover, feasibility of SPAT aided by other optical contrast agents including metallic nanocolloids will also be examined.

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Figure 1.
Schematic of the experimental setup for SPAT measurement of tissue optical absorption spectrum. NDF: neutral density filter.
Figure 2.
Optical absorption spectrum of the contrast agent (IR dye FHI90011S) measured by SPAT.
Figure 3.
Typical photoacoustic signal measured from the vessel. Inset shows the typical photodiode signal of the laser pulse.
Figure 4.
Optical absorption spectra of an oxygenated (circle) and a deoxygenated (square) canine blood specimens obtained with the SPAT system in comparison with the reference (solid curves). The blood oxygen saturation ($SO_2$) were 1.00 for the oxygenated blood specimen and 0.33 for the deoxygenated blood specimen.
Figure 5.
Photoacoustic spectra of an oxygenated (circle) and a deoxygenated (square) canine blood specimens embedded in a scattering medium made from diluted whole milk. The blood oxygen saturation (SO₂) were 0.97 for the oxygenated blood specimen and 0.10 for the deoxygenated blood specimen. (a) and (c) are the PA spectra before correction and (b) and (d) are the PA spectra after correction by introducing the optical contrast agent. Solid curves represent the reference.
Figure 6.
Photoacoustic spectra of an oxygenated (circle) and a deoxygenated (square) canine blood specimens embedded 3-mm deep in a chicken breast tissue. The blood oxygen saturation ($SO_2$) were 0.98 for the oxygenated blood specimen and 0.27 for the deoxygenated blood specimen. (a) and (c) are the PA spectra before correction and (b) and (d) are the PA spectra after correction by introducing the optical contrast agent. Solid curves represent the reference.