Photoacoustic technique
for assessing optical
scattering properties of
turbid media

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Abstract. Measurement of tissue optical properties im-
pacts both optical diagnostic and therapeutic applications.
Although existing photoacoustic imaging techniques pro-
vide optical absorption contrast, we present a photoacous-
tic technique that demonstrates sensitivity to the optical
scattering coefficient of a turbid medium. By incremen-
ting the distance between a surface illumination spot and a
subsurface absorber and measuring the photoacoustic am-
plitude of the absorber, we can effectively estimate the
Green’s function of light transport in a turbid medium.
Our results for different concentrations of Intralipid indi-
cate that this technique has the ability to distinguish small
changes of the reduced scattering coefficient. It has the
potential to be used for in vivo studies to obtain reduced
scattering coefficients of biological tissues. These findings
will potentially improve the calculation of subcutaneous
fluence in photoacoustic-based techniques and laser do-
simetry studies in live tissues. © 2009 Society of Photo-Optical
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ultrasound; Q-switched lasers; biophotonics; index matching liquid.

In the last decade, there has been tremendous interest in
photoacoustic imaging of soft tissues. When short laser
pulses are fired into a tissue, acoustic pressure waves are gen-
erated from optical absorbers due to thermoelastic expansion.
The acoustic pressure wave or photoacoustic signal that is
related to locally absorbed laser energy of an optical absorb-
ing structure can be detected by an ultrasound transducer.
Spatial resolution of the photoacoustic image is determined by
the ultrasound focus and receiver bandwidth. This photoa-
coustic signal is directly proportional to optical absorption,
aser fluence, and the Gruneisen parameter. The local laser
fluence at the absorber location is determined by a combina-
tion of optical absorption and optical scattering. Current mul-
tiwavelength photoacoustic methods of blood oxygen satu-
ration estimation suffer from unknown laser fluences at
subsurface vessel. By knowing the wavelength-dependent tis-
ue optical scattering properties, laser fluence at the blood
vessel locations may be estimated more accurately to improve
SO2 estimates. In this letter, we propose a photoacoustic tech-
nique for assessing optical scattering properties of tissues. In
contrast to surface reflectance measurements in NIR and OIR
techniques, this method measures the effective fluence at an
absorber located inside the tissue.

In this technique, we focus a small laser spot on to the
surface of a turbid medium. We use a focused ultrasound
transducer to record the photoacoustic signal due to a subsur-
face absorbing structure. The fluence at the absorbing struc-
ture depends on the optical properties of the turbid medium.
While keeping the ultrasound focus on the fixed absorbing
structure, the light spot is laterally translated across the sur-
face. The fluence at the absorbing structure changes with the
distance between the light spot and the absorbing structure.
The resultant photoacoustic signal captured by the transducer
is a function of light-spot translation distance, and the mea-
sured curves of photoacoustic amplitude versus light-spot
translation distance provide an estimate of the Green’s func-
tion of light transport, because the photoacoustic signal is
proportional to local laser fluence.

We developed a unique photoacoustic probe for this pur-
pose. Figure 1 illustrates the design of the probe. It consists of
two focusing lenses (f = 25 and 40 mm), a 10-mm fused silica
prism, a 25-MHz ultrasound transducer (V324-SM, Panametrics-NDT, Waltham, MA, USA), and an index-
matching liquid (Catalog No. 19569, Cargille Labs, Cedar
Grove, New Jersey). The transducer has a 6-mm active aper-
ture and a 12.7-mm focal distance. The index-matching liquid
has a refractive index similar to fused silica (n = 1.46).
The prism, the index-matching liquid, and the ultrasound trans-
ducer are housed in a custom-machined acrylic holder.
The index-matching fluid was held underneath the prism by at-
taching a ~25-μm-thick Saran Wrap membrane to the bot-
tom of the holder using double-sided tape. The focusing
lenses above the prism were mounted separately so that it can
be translated along z-axis to form a small focused laser spot at the surface of the turbid medium.

The photoacoustic signal propagating up into the index-matching liquid medium is reflected at the interface to reach the horizontally mounted ultrasound transducer. The maximum photoacoustic signal is produced when the ultrasound focus is confocal with the laser beam [Fig. 1(a)]. A human hair attached to a thin plastic plate with a 4 × 2-cm opening was mounted parallel to the y-axis. It was aligned with the ultrasound focus via an XYZ stage by maximizing the ultrasound pulse echo signal.

The optical index-matching liquid enables nonrefracted light delivery through the fused silica prism to the turbid medium while providing a suitable propagation medium for the photoacoustic signal. The acoustic impedance and acoustic speed values are close to that of soft tissue or water, fortuitously minimizing reflections or distortions at the liquid-tissue boundary (Table 1). Only 1% intensity reflection coefficients for normal incidence ultrasound are expected. Additionally, some minimal losses due to the fine membrane will occur.

![Fig. 1](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics on 9/15/2018)

**Fig. 1** Photoacoustic probe. L1: lens f=25 mm, optional L2: lens f =40 mm with radius of curvature=distance between L2 surface and optical focus, L: pulsed laser beam, P: 10 mm right-angle quartz silica prism, IML: index-matching liquid, UST: ultrasound transducer, M: fine membrane, A: Absorbing structure located in a turbid media. (a) Laser beam confocal with the ultrasound focus and (b) laser beam after translocation.

Because the acoustic impedances of the optical index-matching liquid and fused silica glass are quite different, the optimal reflection of acoustic energy can be attained at the glass-liquid interface. Our calculation shows that when the ultrasound incident angle (with respect to normal of the prism surface) is >23°, 100% of ultrasound will be reflected. The ultrasound incident angle at the glass-liquid interface is 45 ± 13.4°; hence, all of the photoacoustic signal will be reflected toward the transducer. The speed of sound in the index-matching liquid is close to the speed of sound in water. The measured acoustic attenuation in the index matching liquid is 2.7 ± 0.1 dB/cm at 25 MHz, only slightly higher than water.

Initially, we used pulse echo ultrasound to position the target absorber (the hair) at the ultrasound focus by maximizing the output signal that was first amplified by an ultrasound pulse receiver (5073PR, Panametrics-NDT, Waltham, Massachusetts) and then captured by a digital oscilloscope (DPO7054, Tektronix, Beaverton, Oregon). After receiving the maximum signal, the translation stage was locked and the ultrasound receiver was changed to receive mode. A horizontal laser beam (532 nm, Nd:YAG) deflected downward by a prism and lens was attached to an XYZ stage. The laser beam was focused on to the fine membrane by translating the lens along the z-axis. The optical focus was set slightly below the membrane surface. The prism and lens were translated along the x-axis until the maximum photoacoustic signal was observed. At this stage, the laser beam and ultrasound focus are confocal. In addition to a strong photoacoustic signal from the hair, we observed a weak photoacoustic signal from the fine membrane. The depth of the human hair was determined by obtaining the time delay between the photoacoustic signal of the human hair and the fine membrane. The measured depth from the fine membrane to the human hair was 2.05 mm.

As the reduced scattering coefficient \(\mu'_r\) for Intralipid® varies from batch to batch, we employed an oblique-incidence diffuse reflectance technique to calculate \(\mu'_r\) at 532 nm for our stock. We added known Intralipid concentration to water in the tank to produce a predetermined \(\mu'_r\) value. Figure 2 illustrates the normalized photoacoustic signal as a function of lateral light-spot translocation distance for various \(\mu'_r\) values. It can be seen that this method shows clearly separable curves for different \(\mu'_r\) values. As \(\mu'_r\) increases, curves get closer to each other. We demonstrated that our technique is capable of dis-

**Table 1** Optical and acoustic properties of fused silica, index matching liquid, and water. Subscripts L and T represent the longitudinal and transverse acoustic waves in the fused silica.

<table>
<thead>
<tr>
<th></th>
<th>Refractive index</th>
<th>Acoustic impedance (MRayl)</th>
<th>Speed of sound (mm/μs) at 25 MHz</th>
<th>Attenuation (dB/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fused silica</td>
<td>1.46</td>
<td>12.9</td>
<td>5.848</td>
<td>0.006</td>
</tr>
<tr>
<td>Index matching liquid</td>
<td>1.46</td>
<td>12.0±0.003</td>
<td>1.44±0.04</td>
<td>2.7±0.1</td>
</tr>
<tr>
<td>Fused silica</td>
<td>1.33</td>
<td>1.51±0.003</td>
<td>1.51±0.03</td>
<td>1.38±0.02</td>
</tr>
</tbody>
</table>

![Fig. 2](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics on 9/15/2018)

**Fig. 2** Normalized photoacoustic signal as a function of lateral translocation distance for different \(\mu'_r\). Error bars represent the standard deviation of 100 measurements.
tiguous changes of $\mu'_s$ in increments of 1 cm$^{-1}$, however, considering the small error bars for measured points, much finer resolution may be possible, and will be signal-to-noise dependent. Although we used 532 nm for this study due to laser availability, the $\mu'_s$ values we used in this experiment are smaller than the $\mu'_s$ values for the majority of biological tissues at that wavelength. But in the near-infrared region, $\mu'_s$ values of biological tissues are smaller and close to the values we used in the experiment.

Because the ultrasound transducer focus is 12.7 mm, the maximum depth between the membrane and the absorber is limited (<3 mm) by design. Light transport in this region is given by the radiative transport equation. We need transport-regime analytical models or Monte Carlo simulations to estimate optical parameters from these curves. For target depths beyond 3–4 mm, we will be able to use analytical equations based on the diffusion approximation. In future studies, we will employ an appropriate method to extract optical properties from the above curves.

To obtain a strong photoacoustic signal, we used a fraction of a millijoule pulse energy over ~200-µm-diam focused light spot. The estimated fluence of ~1 J/cm$^2$ at the surface is above the ANSI exposure limitations. Several alternatives could be used to spread the laser fluences over a broad area to satisfy the ANSI limits. Instead of using a single high-energy pulse, we could use a series of pulses with low laser energy and average the signals. Another possibility is to use a ring illumination pattern such that the center of the ring will be coaligned with the absorbing structure. Otherwise, the size of the laser-ocusing spot can be increased to spread the laser fluence over a broader range, at the expense of blurring the effective Green’s functions. This, however, may be acceptable as long as optical properties can still be estimated.

In this technique, the laser spot was translated only a short distance along the x-axis (<1 mm) to get the normalized photoacoustic signal. Hence, this method is suitable for samples with a small surface area. That is a great advantage over existing NIR and OIR methods. Even though this method presently uses only phantoms, it has the potential to be used for in vivo studies, where a small blood vessel can be isolated as an absorbing structure. However, more blood vessels (absorbing structures) present in the region (spaced closer than an acoustic focal width) may adversely affect the results.

To date, we know of no method that measures the internal fluence of a turbid medium to obtain its optical parameters. The measured curves can be considered an estimate of the normalized Green’s function of light transport. Fitting the measured curves to models of light transport may facilitate estimation of the scattering and absorption coefficients of tissue. Such predictions may improve existing estimates of absolute absorber concentrations in photoacoustic imaging, including photoacoustic-based oxygen saturation imaging and laser dosimetry. Our current study is limited to an isotropic turbid medium with a single laser source. Speed-of-sound inhomogeneities will lead to receive-focusing aberrations that may introduce a source of variance in the measured scattering curves, although this is estimated to be a minor effect over the small tissue volumes interrogated. Optical heterogeneities will also be a source of error; however, we may be able to obtain optical parameters of complex tissue structures with multiple sources and detectors. Future work may include inverse problem attempting to estimate absorption and scattering inhomogeneities due to multiple optical sources and photoacoustic detection.

In summary, we described a novel photoacoustic concept to differentiate turbid media with different scattering coefficients. In previous attempts of using photoacoustic methods to demonstrate sensitivity to optical scattering, an ultrasound transducer was mounted on the side of a cuvette or opposite the side of the light delivery. Such a setup is not feasible for measuring an optical scattering coefficient of live tissues. This is the first report to our knowledge of using photoacoustic methods that can be applied to demonstrate sensitivity to optical scattering in the reflection mode. We developed a unique probe to implement the above concept, which allows unparalleled flexibility in photoacoustic light delivery. The results obtained for different concentrations of Intralipid clearly demonstrate that this technique can be employed to distinguish small changes in the value of $\mu'_s$. In future studies, we will compare these curves with light-transport models to obtain optical properties of the turbid medium. Such findings have the potential to quantitatively image and estimate tissue properties.

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