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Measurements of wavelength dependent scattering and backscattering coefficients by low-coherence spectroscopy

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Abstract. Quantitative measurements of scattering properties are invaluable for optical techniques in medicine. However, noninvasive, quantitative measurements of scattering properties over a large wavelength range remain challenging. We introduce low-coherence spectroscopy as a noninvasive method to locally and simultaneously measure scattering μ_s and backscattering μ_b coefficients from 480 to 700 nm with 8 nm spectral resolution. The method is tested on media with varying scattering properties (μ_s = 1 to 34 mm⁻¹ and μ_b = 2.10⁻⁶ to 2.10⁻³ mm⁻¹), containing different sized polystyrene spheres. The results are in excellent agreement with Mie theory. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3553005]

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Quantitative determination of the optical properties of tissue is invaluable in biomedical optics. The majority of optical diagnostic techniques rely on the spectral absorption and scattering properties of tissue, which provide information on its composition and structure. The same optical properties are of essential importance for the development and optimization of optical therapeutic techniques. However, despite the existence of many spectroscopic methods, it is still a challenge to do noninvasive, quantitative measurements of the absorption and scattering properties in vivo over a large wavelength range.

Recently, we introduced low-coherence spectroscopy (LCS) to do quantitative and localized measurements of absorption coefficients μ_a over a wavelength range of 480 to 700 nm with a spectral resolution of 8 nm (all wavelength dependent parameters in this paper will be denoted by a boldfaced character).

In this study, we use LCS to quantitatively and simultaneously measure scattering μ_s and backscattering μ_b coefficients on a wide range of scattering media (μ_s = 1 to 34 mm⁻¹ and μ_b = 2.10⁻⁶ to 2.10⁻³ mm⁻¹)). Thereby, we demonstrate new opportunities for noninvasive scattering property measurements. In vivo measurements of the quantitative value of μ_s and μ_b can assist in differentiating between tissue types and modeling of light-tissue interactions. The spectrally resolved information of μ_s and μ_b gives additional valuable information such as the power dependency of μ_s on wavelength and wavelength dependent oscillations in μ_b, which have shown to be related to tissue morphology.

Whereas extensive study on tissue (back)scattering has been performed in the areas of light scattering spectroscopy and angle-resolved low-coherence interferometry, these studies lack quantification of μ_s and μ_b, since their primary aim has been to retrieve the size of the scattering particles. Quantification of μ_s and μ_b has been shown in optical coherence tomography studies, but these studies were limited to the measurement of μ_s and μ_b averaged over the bandwidth of the spectrum, i.e., no spectral information was obtained. Moreover, in these studies, quantitative agreement with theory is rarely obtained for highly scattering media, due to multiple scattering contributions to the signal. Other (diffuse) reflectance spectroscopy techniques are able to measure μ_s and the reduced scattering coefficient μ_s' but this requires additional information on the scattering anisotropy g to obtain μ_b. Thus, compared to the existing methods for scattering property measurements, LCS offers the unique possibility for a combination of simultaneous, quantitative, and spectrally resolved measurement of μ_s and μ_b. Therefore, these measurements will assist in a more complete, and likely more accurate, characterization of the tissue of interest. In addition, like other low coherence interferometry techniques, LCS measures a controlled and confined volume, which is important when measuring local optical properties in an often inhomogeneous tissue.

Using LCS, we measured μ_b and μ_s of aqueous nonabsorbing suspensions of different sized polystyrene spheres and validated our results with Mie theory. Therefore, we measured backscattered power spectra S(ℓ) at controlled geometrical path lengths ℓ of the light in a sample. Our LCS system, which is described in detail in Ref. 1, consists of a Michelson interferometer and is optimized for 480 to 700 nm. The geometrical round trip path length ℓ (ℓ = 0 to 2 mm, with ℓ = 0 the sample surface) is controlled by translating the reference mirror, in steps of 27 μm. By translating the sample, focus tracking of the 64 μm² spot size in the sample is achieved. Around ℓ, the signal is modulated by scanning the piezo-driven reference mirror (23 Hz) resulting in a scanning window of Δℓ ≈ 44 μm. The optical power at the sample is 6 mW.

A multimode fiber (θ = 62.5 μm) guides the reflected light from both arms to a photodiode. Signal processing after acquisition, which is described in detail in Ref. 1, results in averaged spectra S(ℓ) with 8-nm resolution (≈500 averaged per ℓ, to avoid any spectral modulations on S(ℓ) caused by interference between scattering particles). We describe S(ℓ) with a single exponential decay model (Ref. 2) S(ℓ) = S_0 · T · Δℓ · μ_b,NA · exp(−μ_s · ℓ), where S_0 is the source.
power spectrum and $T$ is the system coupling efficiency. When
$S(\ell)$ is dominated by a single backscattered light, $\mu_T$ is the
attenuation coefficient of the sample and $\mu_s$ equals $\mu_T$ for nonabsorbing
samples (this study). The system dependent parameters will be
denoted by $\xi = S_0 \cdot T \cdot \Delta f$. The spectra $S(\ell)$ are collected over
the detection numerical aperture (NA) of the system, therefore,
we define the measured backscattering coefficient $\mu_{b,NA}$ as the
product of $\mu_s$ and the phase function $p(\theta)$, integrated over the
solid angle of the NA in the medium:

$$\mu_{b,NA} = \mu_s \cdot 2\pi \int_{\theta = -\xi - NA}^{\theta = \pi - NA} p(\theta) \cdot \sin(\theta) \cdot d\theta. \quad (1)$$

We measured the wavelength dependent point spread function in
the medium and derived the NA (ranging from 0.035 to 0.045
between 480 to 700 nm) from the resulting Rayleigh length of
the system. The terms $\xi$, $\mu_{b,NA}$, and $\mu_s$ are obtained by fitting a
two-parameter (amplitude and decay, respectively) exponential
function to $S(\ell)$ versus $\ell$. Uncertainties are estimated by the 95% confidence intervals (c.i.) of the fitted parameters.

The model is fitted to the measured $S(\ell)$ up to a path length in the sample of five times the mean free path ($5\mu_s$ from Mie theory at 480
nm, varying from 100 to 1950 $\mu$m). Spectra acquired from $\ell < 50 \mu$m suffer from boundary artifacts and are therefore ex-
cluded from the fits. Prior to fitting the model to $S(\ell)$, a noise
level is subtracted from $S(\ell)$, which is the sum of the dc spectra of
the sample and reference arm. Now, $\mu_{b,NA}$ can be calculated from
the fitted amplitude $\xi$ and $\mu_{b,NA}$, if $\xi$ is determined in a sep-
erate calibration measurement in which $\mu_{b,NA}$ is exactly known
from Mie theory and Eq. (1). To this end, we used National Institu-
tate of Standards and Technology (NIST)-certified polystyrene
spheres of $\phi = 409 \pm 9$ nm (diameter $\pm$ SD, Thermo Scientific,
USA). The obtained $\xi$ was used to determine $\mu_{b,NA}$ in subse-
quent measurements.

In our Mie calculations, we used wavelength dependent re-
fractive indices of water and polystyrene and integrated over the
distance of the spheres (2$\phi$SD), given by the manu-
ufacturer. Brownian motion of the polystyrene spheres causes
Doppler broadening of the measured LCS spectra. For adequate
comparison, we convolved the Mie spectra with a Lorentzian,
with a linewidth of 5 to 13 nm, depending on the sphere size-
dependent Doppler frequency distribution of the Brownian mo-
tion of the spheres, similar to our analysis in Ref. 1.

Figure 1(a) shows LCS measurements (dots) of $\mu_s$ for
four aqueous suspensions of different sized NIST-certified
polystyrene spheres: 0.071% with $\phi = 409 \pm 9$ nm, 0.048% with
$\phi = 602 \pm 6$ nm, 0.038% with $\phi = 799 \pm 9$ nm, and 0.033% with
$\phi = 1004 \pm 10$ nm, which lie within the range of scatterer sizes
in biological cells. The sphere concentrations, indicated in vol-
ume percentages, were chosen such that $\mu_s$ was approximately
equal for all samples ($\sim$1.5 mm$^{-1}$ at 600 nm). The LCS mea-
surements agree within 0.2 mm$^{-1}$ of $\mu_s$ from Mie theory
(thick solid lines) over the entire wavelength range of 480 to 700
nm. The scattering coefficient has a power dependence on
wavelength, with different scatter power for different particle
sizes. We also measured the attenuation coefficient of water,
which, as expected, is $\sim$0 mm$^{-1}$ for all wavelengths.

Figure 1(b) shows the LCS measurements (dots) of $\mu_{b,NA}$ on a
logarithmic scale for the polystyrene suspensions, after measur-
ing $\xi$ on the 409-nm sample. The error bars in this graph are on
the same order of magnitude as the marker size. The $\mu_{b,NA}$ differ
over an order of magnitude between samples, since the phase
function changes considerably with sphere size. The measured $\mu_{b,NA}$ are in agreement with Mie theory (thick solid lines), show-
ing the characteristic sphere size dependent oscillations. The $\mu_{b,NA}$
of water shows no pronounced spectral features, which
implies that our calibration method was applied correctly. We
attribute the small differences between measurements and Mie
calculations to uncertainties in particle size distribution and
refractive index that were used as Mie-input (depending on wave-
length, a 1% change in the polystyrene refractive index results
in a 11 to 14% change in $\mu_s$ and a 11 to 25% change in $\mu_{b,NA}$).

To test the range of validity of the single exponential decay
model to obtain $\mu_s$ and $\mu_{b,NA}$, it is important to also test the
model for media with higher scattering densities. Therefore,
we increased the particle concentration for the 409 nm sample
several times (from 0.071% to 0.950%) and measured $\mu_s$ and
$\mu_{b,NA}$. Figure 2(a) shows that the measured $\mu_s$ agrees with Mie
calculations of $\mu_s$ within 14%, up to values as high as 34 mm$^{-1}$,
which lies well within the range of tissue scattering. In addition,
the measured $\mu_{b,NA}$ is in agreement with Mie theory [(Fig. 2(b)],
except for the two highest volume concentrations, where the
measurement overestimates $\mu_{b,NA}$ at the shorter wavelengths.

The measurements of $\mu_s$ in Figs. 1(a) and 2(a) demonstrate
that disagreement with the Mie calculated values for the highest
volume concentrations (Fig. 2) is only manifested in $\mu_{b,NA}$ and not
in $\mu_s$ (i.e., $\mu_s$ agrees with the Mie calculated $\mu_s$ within the
95% c.i.). For these samples (0.533% and 0.950%), the average
surface-to-surface distance between the spheres is comparable
to the wavelength: 760 and 556 nm, respectively. Since the effect
of multiple scattering would be visible in the measured value of both
coefficients, we speculate that another effect may cause this
disagreement, i.e., the total scattered field cannot be treated as
the superposition of the scattered field by the individual particles
(dependent scattering). Our results indicate that for these sphere
concentrations, $\mu_{b,NA}$ is altered to favor more backward than
forward directed scattering. Further study is needed to assess
the influence of the particle phase function and interparticle
distance on the measured $\mu_s$ and $\mu_{b,NA}$.
in nonlayered, homogeneous samples, LCS has the potential to be corrected for tissue absorption. Several methods to separate scattering and absorption contributions to the LCS signal, since the principles of tissue types, such as the value of μ_s, requires calibration on a sample with known μ_b,NA. To obtain μ_s from tissue, the measured μ_s needs to be corrected for tissue absorption. Several methods to separate μ_s and μ_b from a single attenuation profile have been proposed. In addition, the simultaneous measurement of both μ_s and μ_b,NA by LCS may eventually assist in separating scattering and absorption contributions to the LCS signal, since the μ_b,NA is proportional to μ_s but independent of μ_b.

Whereas in this study, the scattering properties are measured in nonlayered, homogeneous samples, LCS has the potential to measure μ_s and μ_b,NA in individual layers of layered media such as human skin. The controlled path length and the confined measurement volume due to the confocality of the system, in principle, allow to measure within a layer of choice, which will be a subject of further study. Even for a confined tissue volume, the μ_b,NA is likely to consist of the contribution of a range of scatterer sizes and therefore, it will not exhibit oscillations as clearly presented in Figs. 1 and 2. Nevertheless, tissue specific spectral features in backscattering have been observed and also the absolute value of μ_b,NA contains information on tissue type.

In conclusion, we present quantitative and wavelength dependent measurements of scattering and backscattering coefficients from polystyrene sphere suspensions. Our method applies for a broad range of sphere sizes and particle densities, and is in excellent agreement with Mie theory up to scattering coefficients as high as 34 mm⁻¹. LCS measures μ_s and μ_b simultaneously, over a large wavelength range and with good spectral resolution. The combined wavelength dependent information of μ_s and μ_b is likely to assist in more accurate tissue characterization in tissue optics.

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References


Fig. 2 LCS (dots) and Mie (thick solid lines) results for (a) scattering coefficients μ_s and (b) backscattering coefficients μ_b,NA for six concentrations of 409-nm polystyrene sphere suspensions. Error bars, representing the 95% c.i. of the fitted values, may fall behind data points. The μ_b,NA were calibrated using the 0.071% sample.

The presented results show that LCS enables sample characterization based on absolute values of μ_b,NA and μ_b, which the scatter power in μ_s and oscillations in μ_b,NA. This very combination of optical properties is characteristic for particle or tissue type and therefore offers new opportunities for tissue characterization. Clinical studies have been reported where the measurement of only one parameter was not sufficient to differentiate between tissue types, such as the value of μ_s for measuring (morphological) changes between grades of urothelial carcinoma of the bladder. For these studies, the measurement of both μ_s and μ_b,NA by LCS may assist in better differentiation because low contrast in μ_s can be accompanied by high contrast in μ_b,NA (Fig. 1).

In nonabsorbing samples, μ_b is extracted directly from the measurement and μ_b,NA requires calibration on a sample with known μ_b,NA. To obtain μ_b from tissue, the measured μ_b needs to be corrected for tissue absorption. Several methods to separate μ_s and μ_b from a single attenuation profile have been proposed. In addition, the simultaneous measurement of both μ_s and μ_b,NA by LCS may eventually assist in separating scattering and absorption contributions to the LCS signal, since the μ_b,NA is proportional to μ_s but independent of μ_b.