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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 \( \mu_s \) and backscattering \( \mu_b \) coefficients from 480 to 700 nm with 8 nm spectral resolution. The method is tested on media with varying scattering properties (\( \mu_s = 1 \) to 34 \( \text{mm}^{-1} \) and \( \mu_b = 2.10^{-6} \) to \( 2.10^{-3} \text{mm}^{-1} \)), containing different sized polystyrene spheres. The results are in excellent agreement with Mie theory. The same optical properties are of essential importance for the development and optimization of composition and structure. The majority of optical techniques in medicine lack quantification of \( \mu_s \) and \( \mu_b \) since their primary aim has been to retrieve the size of the scattering particles. Quantification of \( \mu_s \) and \( \mu_b \) has been shown in optical coherence tomography studies, but these studies were limited to the measurement of \( \mu_s \) and \( \mu_b \) 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 \( \mu_s \) and the reduced scattering coefficient \( \mu'_s \), but this requires additional information on the scattering anisotropy \( g \) to obtain \( \mu_b \). 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 \( \mu_s \) and \( \mu_b \). 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 \( \mu_b \) and \( \mu_s \) of aqueous nonabsorbing suspensions of different sized polystyrene spheres and validated our results with Mie theory. Therefore, we measured backscattered power spectra \( S(\ell) \) at controlled geometrical path lengths \( \ell \) 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 \( \ell \) (\( \ell = 0 \) to 2 mm, with \( \ell = 0 \) the sample surface) is controlled by translating the reference mirror, in steps of \( 27 \mu \text{m} \). By translating the sample, focus tracking of the \( 64 \mu \text{m}^2 \) spot size in the sample is achieved. Around \( \ell \), the signal is modulated by scanning the piezo-driven reference mirror (23 Hz) resulting in a scanning window of \( \Delta \ell \approx 44 \mu \text{m} \). The optical power at the sample is 6 mW.

A multimode fiber (\( \phi = 62.5 \mu \text{m} \)) 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 \( S(\ell) \) with 8-nm resolution (\(~500\) averages per \( \ell \), to avoid any spectral modulations on \( S(\ell) \) caused by interference between scattering particles). We describe \( S(\ell) \) with a single exponential decay model (Ref. 2) \[
S(\ell) = S_0 \cdot T \cdot \Delta \ell \cdot \mu_b,\text{NA} \cdot \exp(-\mu_s \cdot \ell),
\]
where \( S_0 \) is the source term.
power spectrum and $T$ is the system coupling efficiency. When $S(\ell)$ is dominated by a single backscattered light, $\mu_s$ is the attenuation coefficient of the sample and $\mu_s$ equals $\mu_b$ for nonabsorbing samples (this study). The system dependent parameters will be denoted by $\xi = S_0 \cdot T \cdot \Delta \ell$. 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 $\mu_b$ and the phase function $p(\theta)$, integrated over the solid angle of the NA in the medium:

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

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 excluded 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 $\xi$, $\mu_{b,NA}$, and $\xi$ is determined in a separate calibration measurement in which $\mu_{b,NA}$ is exactly known from Mie theory and Eq. (1). To this end, we used National Institute 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 subsequent measurements.

In our Mie calculations, we used wavelength dependent refractive indices of water and polystyrene and integrated over the size distribution of the spheres (2$\sigma$SD), given by the manufacturer. 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 motion 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 well within the range of tissue scattering. In addition, the measurements of $\mu_s$ and $\mu_{b,NA}$ are 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 $\mu_{b,NA}$.
to measure in nonlayered, homogeneous samples, LCS has the potential to be corrected for tissue absorption. Several methods to separate μs and μb, 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 μb, NA were calibrated using the 0.071% sample.

The presented results show that LCS enables sample characterization based on absolute values of μb, NA and μs, the scatter power in μs and oscillations in μb, NA. This very combination of optical properties is characteristic for particle or tissue type\textsuperscript{2–7} 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 μb for measuring (morphological) changes between grades of urothelial carcinoma of the bladder.\textsuperscript{10} For these studies, the measurement of both μs and μb, NA by LCS may assist in better differentiation because low contrast in μs can be accompanied by high contrast in μb, NA (Fig. 1).

In nonabsorbing samples, μb is extracted directly from the measurement and μb, NA requires calibration on a sample with known μb, NA. To obtain μb from tissue, the measured μb needs to be corrected for tissue absorption. Several methods to separate μs and μb from a single attenuation profile have been proposed.\textsuperscript{11,12} In addition, the simultaneous measurement of both μs and μb, NA by LCS may eventually assist in separating scattering and absorption contributions to the LCS signal, since the μb, NA is proportional to μs but independent of μb.

Whereas in this study, the scattering properties are measured in nonlayered, homogeneous samples, LCS has the potential to measure μs and μb, 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 μb, 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\textsuperscript{13,14} and also the absolute value of μb, NA contains information on tissue type.\textsuperscript{2}

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 μm\textsuperscript{-1}. LCS measures μs and μb simultaneously, over a large wavelength range and with good spectral resolution. The combined wavelength dependent information of μs and μb is likely to assist in more accurate tissue characterization in tissue optics.

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References


