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Estimation of the scattering coefficients of turbid media using angle-resolved optical frequency-domain imaging

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Abstract

Noninvasive measurements of the scattering coefficients of optically turbid media using angle-resolved optical frequency-domain imaging (OFDI) are demonstrated. It is shown that, by incoherently averaging OFDI reflectance signals acquired at different backscattering angles, speckle noise is reduced, allowing scattering coefficients to be extracted from a single A-line with much higher accuracy than with measurements from conventional OFDI and optical coherence tomography systems. Modeling speckle as a random phasor sum, the relationship between the measurement accuracy and the number of compounded angles is derived. The sensitivity analysis is validated with measurements from a tissue phantom.

Knowledge of the scattering coefficients of biological tissues is important for the development and application of noninvasive optical diagnostic and therapeutic methods. Many different methods have been documented for quantifying the scattering (μ_s), absorption (μ_a), total scattering coefficients ($\mu_t = \mu_a + \mu_s$), and reduced scattering coefficients (μ'_s) noninvasively, using approximations and inversion models of varying complexity. These methods include spatially resolved steady-state diffuse reflectance [1], time-domain diffuse reflectance [2], frequency-domain diffuse reflectance [3], confocal microscopy [4], modulated imaging [5], and optical coherence tomography (OCT) [6]. OCT and its Fourier-domain counterpart, optical frequency-domain imaging (OFDI) [7], are particularly attractive methods, as they allow measurements of heterogeneous structures *in vivo*. With the single-backscattering model, measurements of μ_s or μ_t can be made, depending on whether the effects of absorption can be neglected. Models that account for multiple scattering have been shown to allow measurements of μ_s and g , the anisotropy parameter [8]. The accuracy of scattering coefficient measurements with conventional OCT and OFDI is limited by speckle, however, which greatly reduces the signal-to-noise ratio. Although averaging over many spatial locations can reduce speckle and permit measurements of μ_s with high accuracy [9], this type of averaging reduces the resolution with which differences in scattering coefficients can be resolved. Angle-resolved OFDI was recently demonstrated as a novel method of acquiring OFDI images that allows substantial speckle reduction by averaging the magnitudes of multiple interferometric scans (A-lines) obtained simultaneously from a broad range of backscattering angles [10].

Angle-resolved OFDI signals are acquired in the Fourier domain as a function of the laser source wavelength, which is swept in time. Reconstruction of depth-resolved reflectance profiles is performed separately for signals that correspond to different backscattering angles. As such, the reconstructed signals $I(z_m, \theta_n)$ are functions of the discrete depth variable z_m and the discrete angular variable θ_n ; the latter is measured relative to the incident beam. In the case that only one polarization component of the backscattered field is analyzed, the signal observed

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after background subtraction is a function of the squared modulus of the complex fringe amplitude, $\mathbf{a}_s^\perp(z_m, \theta_n)$:

$$I(z_m, \theta_n) = |\mathbf{a}_s^\perp(z_m, \theta_n)|^2. \quad (1)$$

In our notation, the superscript $\perp$ refers to the detected polarization state. We model $\mathbf{a}_s^\perp(z_m, \theta_n)$ as originating from M scatterers within the spatial volume element centered at z_m , each having a backscattering cross section b and an associated phase term $\Delta_k(z_m, \theta_n)$, where k is a scatterer index:

$$\begin{aligned} \mathbf{a}_s^\perp(z_m, \theta_n) &\propto \exp[-\mu_{\text{eff}} z_m (1 + \sec \theta_n)/2] \\ &\times \sum_{k=1}^M \sqrt{b} \exp[i \Delta_k(z_m, \theta_n)] \\ &= \sqrt{\mu_b} \exp[-\mu_{\text{eff}} z_m (1 + \sec \theta_n)/2] \varepsilon(z_m, \theta_n), \end{aligned} \quad (2)$$

and $\mu_b = Mb$ is the backscattering coefficient. We note that μ_b is distinct from μ_{eff} and was not considered for measurement in this study. The speckle term corresponding to a single-backscattering angle, $\varepsilon(z_m, \theta_n)$, is a random variable due to the random nature of the spatial distribution of scatterers and is defined according to Eq. (2) as

$$\varepsilon(z_m, \theta_n) = \frac{1}{\sqrt{M}} \sum_{k=1}^M \exp[i \Delta_k(z_m, \theta_n)]. \quad (3)$$

The secant within the exponent reflects the difference in distances traveled by photons backscattered at different angles within the scattering medium. The exponential attenuation term μ_{eff} arises from scattering and absorption losses before and after the backscattering event. It has previously been observed that the signal attenuation with depth is greater than can be accounted for by the scattering coefficient alone, a phenomenon that has been attributed to the inverse relationship between collection efficiency and distance from the focus [11]. We model the relationship between μ_{eff} and the actual scattering coefficient μ_s with the following empirical expression: $\mu_s = \alpha_1 \mu_{\text{eff}} + \alpha_2 \mu_{\text{eff}}^2$, where α_1 and α_2 are real constants. We assume that the intensity of scattered light does not vary significantly as a function of angle over the detected range, as is the case for scatterers with small size parameters [12].

The scattering coefficient can be recovered from the log-transformed OFDI signal, $\mathbf{I}_{\log}(z_m, \theta_n)$, using the following linear regression model in conjunction with the parameters α_1 and α_2 :

$$\mathbf{I}_{\log}(z_m, \theta_n) = \log[I(z_m, \theta_n)] = A + B(\theta_n) z_m + \varepsilon_{\log}(z_m, \theta_n), \quad (4)$$

where A is a constant, $B(\theta_n) = -\mu_{\text{eff}}[1 + \sec(\theta_n)]$, $\varepsilon_{\log} = \log(|\varepsilon|^2) - \bar{\varepsilon}_{\log}$, and $\bar{\varepsilon}_{\log}$ is the expectation of $\log(|\varepsilon|^2)$. Subsequently, we employ the approximation $1 + \sec(\theta_n) \approx 2$, which is valid to within 0.1% for $|\theta_n| \leq 5^\circ$.

The accuracy at which the scattering coefficient can be estimated is directly dependent on the standard deviation of $\varepsilon_{\log}$, which we denote $\sigma_{\varepsilon,1}$. When angular compounding is performed

with N uncorrelated samples, the standard deviation of the resulting speckle term $\sigma_{\varepsilon,N}$ is smaller than $\sigma_{\varepsilon,1}$ by a factor of $\sqrt{N}$. It follows from linear regression theory that least-squares estimates of μ_{eff} have a standard deviation σ_{eff} given by [13]

$$\sigma_{\text{eff}}^2 = \frac{\sigma_{\varepsilon,1}^2}{N} \left(\sum_{k=1}^N z_k^2 \right)^{-1}. \quad (5)$$

The error of estimation of the scattering coefficient, σ_{μ} , is related to σ_{eff} by the parameters α_1 and α_2 . In particular, in the case that $\alpha_1 \gg \alpha_2$, the relationship is well approximated by the expression $\sigma_{\mu} = \sigma_{\text{eff}}/\alpha_1$.

In the limit of fully developed speckle where $M \rightarrow \infty$, the random phasor sum in Eq. (3) can be considered as a complex Gaussian random variable with zero mean and unit variance, a result of the central limit theorem. It can be shown by a change of variables that the ensuing probability density function for ε is an exponential distribution with parameter unity [14]. It follows that the log-transformed speckle term, $\varepsilon_{\log}$, has the following distribution:

$$p(\varepsilon_{\log}) = \exp[\varepsilon_{\log} + \bar{\varepsilon}_{\log} - \exp(\varepsilon_{\log} + \bar{\varepsilon}_{\log})]. \quad (6)$$

This distribution, which has no free parameters, has zero mean and variance $\sigma_{\varepsilon}^2 = \pi^2/6$. It allows for the exact calculation of the standard deviation of μ_{eff} in Eq. (5).

To experimentally validate our sensitivity analysis, angle-resolved OFDI measurements were made from tissue phantoms designed to be optically homogeneous. The phantoms were constructed from aqueous agar gel (Sigma) with embedded polymer microspheres of diameter $0.3 \mu\text{m}$ (Duke Scientific). The angle-resolved OFDI system of [10], incorporating an InGaAs line-scan camera, was used to simultaneously detect interference signals from multiple backscattering angles centered at 180° . The wavelength-swept laser had a wavelength range of 130 nm centered at 1295 nm . Angular data representing the range $\pm 5^\circ$, as detected by 125 array elements, was employed for analysis. The transverse scanning range was 4 mm . Axial and transverse resolutions were 8 and $24 \mu\text{m}$ in air, respectively. Reconstruction steps included background subtraction, interpolation to linear k -space, windowing, and the discrete Fourier transform. Signal correlations in the angular domain were found to extend across 0.4° ($1/e^2$ width of the central peak of the autocorrelation function) and were largely eliminated by downsampling by a factor of 5, resulting in 25 angular samples per spatial volume element. Likewise, downsampling by a factor of 3 was performed separately in the axial and transverse dimensions.

An A-line corresponding to a single angular sample from a one-layer phantom constructed with a microsphere density of $8.8 \times 10^{11} \text{ ml}^{-1}$ shows significant speckle [Fig. 1(a)]. As such, it contrasts markedly with the A-line resulting from angular compounding across 25 angular samples [Fig. 1(b)]. The log-transformed signal was observed to be linear for approximately $500 \mu\text{m}$. The deviation from linearity at greater depths can be attributed to the effects of multiple scattering [8]. Within the upper linear range, the probability density function of the residuals obtained from subtracting the log-transformed signal from the linear fit was found to be well described by Eq. (6) (Fig. 2).

Linear regression of log-transformed OFDI signals obtained from tissue phantoms with scattering coefficients ranging from 0.82 to 2.7 mm^{-1} , as calculated by Mie theory, was

employed to derive the calibration parameters $\alpha_1 = 0.47$ and $\alpha_2 = 0.060$. For Mie theory calculations, a value of 1.57 was employed for the microsphere refractive index at 1295 nm. Subsequently, a scattering coefficient estimate of 1.50 mm^{-1} was obtained for a tissue phantom separate from those in the calibration set, with a microsphere density of $8.8 \times 10^{11} \text{ ml}^{-1}$, by averaging the estimates from 2000 sequential A-lines that corresponded to a backscattering angle of 180° . For this phantom, this was deemed the “gold standard” estimate, as it was obtained with high levels of averaging. It is in good agreement with the value of 1.54 mm^{-1} obtained by the Mie theory. The standard error, calculated as the standard deviation of scattering coefficient estimates from different A-lines divided by the gold standard estimate, varied with the extents of angular and spatial averaging. The relationship between the standard error and the number of compounded angles, N , was found to be in excellent agreement with the theoretical one [Eq. (6)]. A similar relationship was obtained between the standard error and the number of averages performed across different A-lines, in the absence of angular compounding (Fig. 3). This result highlights the equivalence between averaging over uncorrelated angular and transverse spatial samples from the standpoint of reducing speckle noise. Of the two averaging methods, angular averaging has the advantage of not degrading the spatial resolution of scattering coefficient measurements.

In summary, we have demonstrated accurate estimation of scattering coefficients using incoherently angularly compounded OFDI signals. The observed estimation errors were well predicted by a sensitivity analysis of the linear regression estimation method that incorporated a statistical model of fully developed speckle. As speckle averaging can be performed across angular samples instead of across spatial samples, angle-resolved OFDI has the potential to provide accurate measurements of the scattering coefficients in heterogeneous biological structures with significantly higher spatial resolution than are possible with conventional OFDI and OCT.

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Fig. 1. Log-transformed OCT signals from a single angular sample corresponding to 180° backscattering and inset angular compounding across 25 angles within the range of $180 \pm 5^\circ$, with corresponding linear fits over the range of $500 \mu\text{m}$ within the tissue phantom.

Fig. 2. Probability density function (PDF) of the residuals from linear fits to log-transformed signals from a single angular sample corresponding to 180° backscattering (circles), with the corresponding theoretical PDF of Eq. (6) (solid curve). The signals were constrained to correspond to points $500\ \mu\text{m}$ from the surface of the tissue phantom.

Fig. 3. Standard error of estimated scattering coefficients as a function of the numbers of compounded angular samples (circles) and distinct A-lines (squares) with the corresponding theoretical values from Eq. (6) (solid curve). The standard error is expressed relative to the gold standard estimate of 1.50 mm^{-1} .