Functional imaging of dye concentration in tissue phantoms by spectroscopic optical coherence tomography

Trude Støren  
Norwegian University of Science and Technology  
Department of Physics  
N-7491 Trondheim, Norway  
E-mail: Tore.Lindmo@phys.ntnu.no

Arne Røyset  
SINTEF Materials Technology  
Department of Applied Physics  
N-7465 Trondheim, Norway

Lars O. Svaasand  
Norwegian University of Science and Technology  
Department of Electronics and Telecommunications  
N-7491 Trondheim, Norway

Tore Lindmo  
Norwegian University of Science and Technology  
Department of Physics  
N-7491 Trondheim, Norway  
E-mail: Tore.Lindmo@phys.ntnu.no

Abstract. We present functional imaging of the concentration of a photodynamic therapy (PDT)-related dye in scattering tissue phantoms based on spatially resolved measurements of optical properties through spectroscopic optical coherence tomography (OCT). Expressions for the OCT signal are developed, enabling estimation of depth-resolved sample optical properties. Based on these expressions, we discuss speckle statistics and speckle correlations of the OCT signal. Speckle noise reduction is performed by spatial filtering and is used to improve accuracy in the estimated optical properties at the expense of spatial resolution. An analytic expression for the precision in the estimated optical properties is derived. This expression shows that axial filtering, and thereby a reduction of axial resolution, gives a larger improvement in precision compared to the same filtering and reduction in the transversal resolution. It also shows that imaging with a shorter coherence length, or a larger numerical aperture, improves precision when the filter length determines the spatial resolution. Good agreement is obtained between experimentally determined and theoretically predicted variance in the estimated attenuation coefficients and dye concentration. Finally, we present guidelines for spectroscopic OCT systems for concentration imaging and discuss application of the method to more realistic phantoms and tissue. © 2005 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1898242]

Keywords: optical coherence tomography; speckle; concentration monitoring; photodynamic therapy; optical properties; functional imaging.

1 Introduction

Optical coherence tomography (OCT) has become a well-established technique for obtaining high-resolution structural images of biological and other semitransparent tissues. The introduction of extremely broadband, highly coherent sources and the development of full-field OCT instruments using affordable white light sources enables the imaging resolution to approach that of histology. This increases the value of OCT as a diagnostic tool and has brought OCT closer to the goal of performing noninvasive optical biopsies.

OCT has recently been expanded to different functional imaging modalities such as Doppler OCT, where velocity in a fluid flow can be determined with high spatial resolution, polarization sensitive OCT for, e.g., imaging of sample birefringence, molecular imaging, and spectroscopic OCT.

Several authors have addressed the challenge of extracting quantitative information on optical properties from scattering samples using low-coherence reflectometry (LCR) and OCT. In 1993 Schmitt et al. studied the relationship between optical properties of a homogeneous scattering sample and the signal measured by LCR. Publications by Pan et al., Schmitt and Knüttel, and Thurber et al. give comprehensive models of the LCR signal detected from a scattering sample. In a recent publication, Khodolykh et al. studied how speckle averaging improves the precision of the estimated attenuation coefficient of tissue.

Using one wavelength channel it is possible to obtain information about only the attenuation in the sample, i.e., the sum of absorption and scattering. Spectroscopic OCT, using two or more wavelengths, enables separation of scattering from absorption, making it possible to monitor the concentration of an absorbing analyte in a scattering sample. This requires knowledge of the difference in absorption of the sample at the probing wavelengths. An application of concentration imaging could be monitoring the diffusion of a topically applied sensitizer prior to light exposure in photodynamic therapy (PDT).

It is well known that OCT images, like all other images obtained using coherent light, contain speckle noise. A study of image formation in OCT shows that speckle is both the information carrier and a source of noise degrading image quality. Speckle noise reduction is performed by spatial filtering and is used to improve accuracy in the estimated optical properties at the expense of spatial resolution.
quality. Much work has concentrated on suppressing speckle noise in OCT images and thereby increasing image SNR. Excellent reviews of the origin of speckle noise and different approaches to speckle noise reduction are given in articles by Schmitt et al., 21 Fercher et al., 22 and Pircher et al. 23 Less work has been done related to the effect and suppression of speckle noise in imaging of optical properties. Care must be taken to ensure that the speckle reduction method does not adversely affect quantitative estimation of the optical properties. 12,16

In this paper, functional imaging of the concentration of an analyte in scattering tissue phantoms is obtained through spatially resolved measurements of optical properties of the phantom by means of spectroscopic OCT. Spatial averaging is used to improve accuracy in the estimated optical properties at the expense of spatial resolution. We present a quantitative analysis of the limitations on axial and transversal resolution in the images due to speckle noise and discuss the practical consequences of these limitations for the usefulness of such functional imaging.

2 Theory

2.1 Sample Optical Properties

Attenuation of light propagating in an absorbing and scattering medium is governed by the total attenuation coefficient

\[ \mu_{\lambda}(r) = \mu_{a,\lambda}(r) + \mu_{s,\lambda}(r), \]

where \( \mu_{a,\lambda}(r) \) and \( \mu_{s,\lambda}(r) \) are the absorption and scattering coefficients of the medium, respectively, generally functions of wavelength and position. It was previously shown that \( \mu_{a} \) can be extracted from OCD measurements as long as the light contributing to the OCT signal is dominated by single-backscattered photons. 12,16,24

Consider a scattering sample containing an absorbing dye having concentration \( C_{\text{dye}} \), e.g., tissue with an applied photosensitizer. As long as the dye is the only absorbing component in the sample, the relationship between dye concentration and sample absorption coefficient \( \mu_{a,\lambda} \) is given by the wavelength-dependent extinction coefficient \( \varepsilon_{a,\lambda} \) as

\[ \varepsilon_{a,\lambda} = \frac{\mu_{a,\lambda}}{C_{\text{dye}}}. \]

Assuming that the scattering coefficients at two wavelengths \( \lambda_1 \) and \( \lambda_2 \) are proportional, and that the parameter \( F \) is given by

\[ F = \frac{\mu_{s,\lambda_1}}{\mu_{s,\lambda_2}}, \]

the dye concentration can be expressed in terms of the attenuation coefficients at the two wavelengths as

\[ C_{\text{dye}}(r) = \frac{\mu_{s,\lambda}(r) - \mu_{s,\lambda}(r)F}{\varepsilon_{a,\lambda_1} - \varepsilon_{a,\lambda_2}F}. \]

The simple form of Eq. (4) is due to the assumption that \( F \) and \( \mu_{s} \) are independent of the dye concentration, i.e., the presence of the dye does not change the scattering properties of the sample. The equation is also limited to the case where the dye under study is the only absorbing component in the sample. When measuring the concentration of a dye in live tissue, these assumptions will no longer necessarily be valid, thus complicating the analysis. For live tissue, other chromophores (e.g., hemoglobin and melanin) may contribute to the absorption, and the wavelength dependence of the scattering will probably not be as simple as assumed in Eq. (3). In spite of these challenges, we expect that as long as a known relationship exists between the scattering and the dye concentration, it will be possible to find an expression for the dye concentration based on measurements of the attenuation coefficient at two wavelengths. Note that in the case of \( \varepsilon_{a,\lambda} = F \varepsilon_{a,\lambda} \), i.e., the ratio between the absorption coefficients at the two probing wavelengths equals the ratio between the scattering coefficients, it is not possible to separate scattering from absorption and thus determine \( C_{\text{dye}} \).

2.2 Model of the OCT Signal Received from a Scattering and Absorbing Sample

Considering a scattering and absorbing sample in a low-coherent interferometer, we now develop expressions for the OCT signal enabling estimation of the depth-resolved attenuation coefficient of the sample. Based on these expressions we discuss speckle statistics and speckle correlations of the OCT signal.

We assume that the interferometer is illuminated by a low-coherent source having a normalized spectral distribution \( S(k) \), where \( k = 2 \pi/\lambda \) is the wave number of the radiation at vacuum wavelength \( \lambda \). For a source of total intensity \( I \), the intensity at wave number \( k \) is \( I(k) = IS(k) = |E(k)|^2 \), where \( E(k) \) is the field. The intensities incident on the sample and reference arms are denoted \( I_{0s} = |E_{0s}|^2 \) and \( I_{0r} = |E_{0r}|^2 \), respectively, and the field returning from the reference arm, at wave number \( k \), is

\[ E_r(z_r,k) = E_{0r}(k) \exp(i2kz_r), \]

where \( z_r \) is the reference arm optical path length. For a reference arm having a reflecting mirror in air, the reference arm optical path length is directly given by the position of the mirror along the optical axis.

The light incident onto the sample is focused at a controlled depth, ensuring that the optical path length in the sample arm, to the focus position, equals the optical path length of the reference arm. The transversal position in the sample (position in the \( xy \) plane) is denoted \( r_z \), and the total field, at wave number \( k \), backscattered from the sample can be expressed as

\[ \begin{align*}
E_s(r_z,z_r,k) &= E_{0s}(k) \int_{-\infty}^{\infty} \int_{S_{xy}} |dr'_zJ(r_z-r'_z,z_r-z_r)| \\
&\times O(r'_z,z_r) \exp(i2kz_r),
\end{align*} \]

where \( z_r \) is the optical path length in the sample arm corresponding to geometrical sample depth \( z_r \). \( dr'_z \) represents the differential surface element for integration over the whole \( xy \) plane \( (S_{xy}) \), and \( J(r_z,z_r) \) is a focus function describing the phase and amplitude of light backscattered from position \( (r_z,z_r) \) compared to backscattering from position \( (0,0) \). The focus function depends on the beam amplitude and phase pro-
In Eq. (7) we have used the definition of the normalized, complex coherence function of the source given by \( \gamma(z) = \int_0^\infty dk S(k) \exp(-ikz) \). The last term in Eq. (7) is the interference part of the detector intensity, and is denoted \( I_{\text{int}} \). We see that \( I_{\text{int}} \) is two times the real part of a convolution in both \( r_i \) and \( z_r \), between the object function and the product of the focus function and the coherence function. Letting \( \otimes \) represent the convolution operation, we write

\[
I_{\text{int}}(r_i, z_r) = 2(I_0 I_\odot) \text{Re} \left\{ I_{\text{det}}(r_i, z_r) \otimes J(r_i, z_r) \gamma(2z_r) \right\}
\]

\( = 2(I_0 I_\odot) \text{Re} \left\{ I_{\text{det}}(r_i, z_r) \right\}. \tag{8}\]

Equations (7) and (8) show that the complex interferogram \( I_{\text{det}}(r_i, z_r) \) at transversal position \( r_i \) and reference mirror position \( z_r \), is the sum of complex coherence functions centered at the scattering positions in the sample. The amplitudes and phases of the coherence functions are modified by the focus function centered at optical depth \( z_r \), and transversal position \( r_i \) in the sample. The interferogram will thus have contributions from a probing volume (voxel) in the sample limited transversally by the focal width \( l_i \). Axially, the voxel will be limited by the shortest of the depth of focus \( d_f \) and the coherence length \( l_c \) of the source. For a Gaussian beam, the focal width and depth of focus are related to the wavelength and the \( 1/e^2 \) beam divergence angle in the sample. For a Gaussian beam, the focal width and depth of focus are related to the wavelength and the \( 1/e^2 \) beam divergence angle in the sample. For a Gaussian beam, the focal width and depth of focus are related to the wavelength and the \( 1/e^2 \) beam divergence angle in the sample.

The total detected intensity is found by integrating \( I_{\text{det}}(k) = |E_i(k) + E_r(k)|^2 \) over all \( k \) and will be a function of reference arm optical path length \( z_r \), and transversal probing-light position \( r_i \):

\[
I_{\text{det}}(r_i, z_r) = I_r + I_s(r_i, z_r) + 2 \text{Re} \left\{ E_0 E_0^* \int \int dz_i dr_i' \text{O}(r_i', z_r) \right\} \times J(r_i - r_i', z_r) \gamma^2(z_r - z_r).
\]

In Eq. (7) we have used the definition of the normalized, complex coherence function of the source given by \( \gamma(z) = \int_0^\infty dk S(k) \exp(-ikz) \). The last term in Eq. (7) is the interference part of the detector intensity, and is denoted \( I_{\text{int}} \). We see that \( I_{\text{int}} \) is two times the real part of a convolution in both \( r_i \) and \( z_r \), between the object function and the product of the focus function and the coherence function. Letting \( \otimes \) represent the convolution operation, we write

\[
I_{\text{int}}(r_i, z_r) = 2(I_0 I_\odot) \text{Re} \left\{ O(r_i, z_r) \otimes J(r_i, z_r) \gamma(2z_r) \right\}
\]

\( = 2(I_0 I_\odot) \text{Re} \left\{ I_{\text{det}}(r_i, z_r) \right\}. \tag{8}\]

where \( r_s(r_i, z) \) is the complex random field reflectivity of the sample, while the exponential factor describes the two-way attenuation in the sample according to the Beer-Lambert law. The factor 2 is due to the field propagating to depth \( z \) and back to the sample surface, and the field attenuation coefficient is half the intensity attenuation coefficient \( \mu_r \). In these and the following expressions, the subscript \( \lambda \) denotes the center wavelength of the probing light.

In OCT, it is common to record the envelope \( A_\lambda(r_i, z) \) of the interferogram. While the interferogram of Eq. (8) has Gaussian statistics, the envelope of the interferogram is a Rayleigh-distributed random function. From Eq. (8) we see that if the exponential factor in the object function of Eq. (8) is slowly decaying compared to the axial speckle size, the envelope of the interferogram averaged over the speckle correlation length, given by the coherence length in the sample, is the envelope of the object function averaged over the same length. The ensemble average \( \langle A_\lambda(r_i, z) \rangle \) of the envelope is thus

\[
\langle A_\lambda(r_i, z) \rangle = G_\lambda(|r_s(r_i, z)|) \text{exp} \left\{ - \int_0^z \mu_r(r_i, z') dz' \right\}, \tag{10}\]

where \( r_s(r_i, z) \) is the complex random field reflectivity of the sample, while the exponential factor describes the two-way attenuation in the sample according to the Beer-Lambert law. The factor 2 is due to the field propagating to depth \( z \) and back to the sample surface, and the field attenuation coefficient is half the intensity attenuation coefficient \( \mu_r \). In these and the following expressions, the subscript \( \lambda \) denotes the center wavelength of the probing light.
where $G^\lambda$ is proportional to the intensity of the light incident in the interferometer, the envelope of the coherence function, and gain factors in the detection system.

When recording 2-D cross-sectional OCT images we use the average envelope of $m$ transversally displaced A scans as image function $P_{m,\lambda}$. In this paper, we do not record 3-D data and will in the following denote the transversal direction as $x$ for simplicity. The image function is defined as

$$P_{m,\lambda}(x,z) = \frac{1}{m} \sum_{i=1}^{m} A_\lambda(x_i,z),$$

(11)

where $x$ is the average of the set $\{x_i\}$. The transversal displacement for each A scan is denoted $\Delta x = |x_i - x_{i-1}|$. While, for an envelope dominated by a speckle signal, $P_1$ is Rayleigh distributed, $P_{m>1}$ will have a gamma distribution, and in the limit of large $m$ become Gaussian distributed.\(^2\)\(^3\)\(^4\) Since the image function is a linear function of the OCT envelope, it is straightforward to find the statistical properties of the image function from the statistical properties of the OCT envelope. For all $m$, the ensemble average of the image function is equal to the transversal average of the envelope given in Eq. (10), while its variance is reduced by $m$ for statistically uncorrelated A scans, obtained when $\Delta x$ is larger than the transversal correlation length $l_z$. Since $P_1$ is Rayleigh distributed with mean value $\langle A \rangle$, its variance is given by\(^2\)\(^5\)

$$\sigma^2_{P_1} = \left(\frac{4}{\pi} - 1\right) \langle A \rangle^2.$$

(12)

### 2.3 Estimating Optical Properties and Dye Concentration

We now develop equations used for estimating the attenuation coefficient and thus the dye concentration of a scattering and absorbing sample based on measurements of the depth-resolved envelope at two probing wavelengths. The variance in the estimated dye concentration is dominated by speckle noise, and we derive equations describing how spatial averaging improves precision in the estimated attenuation coefficients and dye concentration.

Taking the natural logarithm of Eq. (10) and differentiating, we obtain

$$\frac{d}{dz} \ln(A_\lambda(x,z)) = \frac{d}{dz} \left[ \ln(\langle r_\lambda(x,z) \rangle) \right] - \mu_{i,\lambda}(z)$$

(13)

when we assume that $G^\lambda$ is independent of depth. The scattering coefficient of a sample is related to its field reflectivity through the relation\(^2\)\(^9\)

$$\mu_{x,\lambda}(x,z) \approx \langle |r_\lambda(x,z)|^2 \rangle.$$

(14)

We see that when $\mu_{x,\lambda}(x,z)$ and thus $\langle |r_\lambda(x,z)| \rangle$ is constant or slowly changing as a function of depth, the first term on the right side of Eq. (13) can be neglected, and we are left with the depth-resolved attenuation coefficient of the sample. Substituting the average envelope with the image function we thus get an estimate for the spatially resolved total attenuation coefficient

$$\hat{\mu}_{i,\lambda}(x,z;m) = - \frac{d}{dz} \left[ \ln (P_{m,\lambda}(x,z)) \right],$$

(15)

where the hat over the attenuation coefficient indicates the estimate. We return to the field-reflectivity term in the discussion of experimental results.

Due to the nonlinear relationship between the image function and the estimator for the attenuation coefficient, we used simulations to study the statistical properties of the estimator. For the simulation, we create statistically independent data sets representing the OCT envelope by drawing from a Rayleigh distribution. The simulations show that when the image function $P_{m,\lambda}$ is an average of $m$ statistically independent realizations of the OCT envelope, the mean value of the logarithm of the image function is $\langle \ln P_m \rangle = \ln(A) - K_1/m$, while the variance is $\sigma^2_{\ln P_m} = K_2/m$. The constants $K_1$ and $K_2$ in these empiric relations were found to be $K_1 = 0.167$ and $K_2 = 0.41$. We see that while the mean value of $\ln P_m$ depends on the average OCT envelope, the variance is independent of the average OCT envelope. When the envelope mean value has the form of Eq. (10), the estimator of Eq. (15) is an unbiased estimator for the attenuation coefficient since the last term in the empiric expression for $\langle \ln P_m \rangle$ is constant with respect to sample depth, and thus vanishes in the differentiation.

To obtain an accurate spatially resolved estimate of the sample optical properties, averaging is necessary. Due to the nonlinearity in the estimator introduced by the logarithm, we choose to carry out the averaging prior to taking the logarithm. We thus convolve the image function with filter functions $h_x(z)$ and $h_z(x)$ in the axial and transversal directions, respectively. Transversally this averaging is equivalent to the averaging inherent in the definition of the image function for $m>1$. Depending on the transversal sampling rate of the recorded data and the width of the transversal filter function it might be useful to combine a reduction of the data set using an image function having $m>1$ with transversal averaging through convolution. In the case of a constant or slowly varying field reflectivity, a speckle-averaged estimate of the spatially resolved attenuation coefficient is thus given by

$$\hat{\mu}_{i,\lambda}(x,z;l_z,L_z) = - \frac{d}{dz} \ln \left[ P_{m,\lambda}(x,z) \ast h_x(x) \ast h_z(z) \right]$$

$$= - \frac{d}{dz} \ln \left[ \int_{-\infty}^{\infty} dx' \int_{-\infty}^{\infty} dz' \right. $$

$$\times P_{m,\lambda}(x',z')h_z(x-x')h_x(z-z')$$

$$\left. \times P_{m,\lambda}(x,z) \right]$$

(16)

where $P_{MN,\lambda}$ represents an image function averaged over $M$ and $N$ statistically independent speckle realizations in the transversal and axial directions, respectively. The transversal and axial filter functions have characteristic lengths $L_z$ and $L_x$, respectively (typically full width at half maximum of the filter functions). Thus, $M$ and $N$ can be approximated by
Essential to measurements of sample optical properties is the precision by which these properties can be determined. We therefore developed an expression for the variance \( \sigma_{\hat{\mu}_t}^2 \) in the estimated attenuation coefficient of Eq. (16). Using the chain rule for differentiation together with the definition of the differential operator, the estimator can be expressed

\[
\hat{\mu}_{t,\lambda}(x,z;L_z,L_t) = -\frac{1}{P_{MN,\lambda}(x,z)} \frac{dP_{MN,\lambda}(x,z)}{dz} - \frac{n \rho_{\Delta P_{MN,\lambda}}(\Delta z)}{\Delta z P_{MN,\lambda}(x,z)^2}.
\]

From this expression, the variance of the estimator can be found:

\[
\sigma_{\hat{\mu}_t}^2 = \langle (\hat{\mu}_t) \rangle^2 - \langle \hat{\mu}_t \rangle^2 = \frac{1}{\Delta \sigma_{\hat{\mu}_t}^2} \frac{\Delta z^2}{\langle P_{MN,\lambda}(x,z) \rangle^2} \left( \frac{P_{MN,\lambda}(x,z+\Delta z)-P_{MN,\lambda}(x,z)}{\Delta z} \right)^2 - \langle \hat{\mu}_t \rangle^2.
\]

Expressing the averaged image function as a sum of its mean value and a varying part, \( P_{MN,\lambda}(x,z) = \langle P_{MN,\lambda}(x,z) \rangle + \Delta P_{MN,\lambda}(x,z) \), and using the fact that the mean value of the image function equals the mean value of the OCT envelope given in Eq. (10), we obtain the following expression:

\[
\sigma_{\hat{\mu}_t}^2 = 2C_{P_{MN,\lambda}}^2 \lim_{\Delta z \to 0} \frac{1 - \rho_{\Delta P_{MN,\lambda}}(\Delta z)}{\Delta z^2},
\]

where \( C_{P_{MN,\lambda}} = C_{A,\lambda}/\sqrt{MN} = [(4/\pi - 1)/MN]^{1/2} \) is the speckle contrast of the averaged image function, and \( \rho_{\Delta P_{MN,\lambda}}(\Delta z) = \rho_{P_{MN,\lambda}}(\Delta z) \) is the autocorrelation of the averaged image function in the axial direction. According to signal processing theory, \( \rho_{P_{MN,\lambda}}(\Delta z) \) is found by convolving \( \rho_{P_{mn,\lambda}}(\Delta z) \), the correlation function for \( P_{m,\lambda}(x,z) \) in the axial direction, with the autocorrelation of the filter function \( h_z \). It can be shown that as long as the \( m \) A scans averaged to obtain the image function are statistically independent, \( \rho_{P_{mn,\lambda}}(\Delta z) = \rho_{A}(\Delta z) \) is the autocorrelation of the OCT envelope given by the autocorrelation of the envelope impulse response \( h_A(z) = |\gamma(2z)| \). Sources having a Gaussian coherence function and using a Gaussian filter function \( h_z(\Delta z) = \exp[-4\ln2(2\Delta z^2/L_z^2)] \), in Eq. (16) we obtain

\[
\rho_{P_{MN,\lambda}}(\Delta z) = [h_A(\Delta z) \otimes h_A(\Delta z)] \otimes [h_z(\Delta z) \otimes h_z(\Delta z)]
\]

\[
= \exp\left[-4\ln2 \frac{\Delta z^2}{2L_z^2} \right],
\]

\[
= \exp\left[-4\ln2 \frac{\Delta z^2}{2L_z^2} \right].
\]

where the approximation is valid for \( L_z \approx L_t \). Inserting the expression for the speckle contrast into Eq. (20) together with a series expansion in \( \Delta z \) of the approximated \( \rho \) from Eq. (21) leads to the following expression for the variance of the attenuation-coefficient estimator:

\[
\sigma_{\hat{\mu}_t}^2 = 4\ln2 \left( \frac{4}{\pi} - 1 \right) \frac{L_zL_t}{L_z^2L_t} = K \frac{l_c}{L_z^2L_t},
\]

where all parameters in the last term are geometrical lengths in the sample. As expected, the expression shows that there is a trade-off of transversal and axial resolution against precision in the determined optical properties. More interesting is the important result that according to Eq. (22), the reduction in speckle noise is more efficient when averaging axially than transversally. The origin of this asymmetry is the differentiation in the axial direction, which amplifies high-frequency spatial noise, thus yielding a more efficient averaging in the axial direction than in the transversal direction when using the same filter length.

Using two probing wavelengths, the attenuation coefficients estimated using Eq. (16) can now be used in Eq. (4) to obtain an unbiased estimator for the dye concentration, \( \hat{C}_{dye}(x,z;L_z,L_t) \), in a scattering sample containing an absorbing dye:

\[
\hat{C}_{dye}(x,z;L_z,L_t) = \frac{\hat{\mu}_{t,\lambda}(x,z;L_z,L_t) - F \hat{\mu}_{t,\lambda_2}(x,z;L_z,L_t)}{\epsilon_{a,\lambda_1} - F \epsilon_{a,\lambda_2}}.
\]

The variance of the estimated dye concentration \( \sigma_{\hat{C}_{dye}}^2 \) is given as a function of the variances of the estimated attenuation coefficients:

\[
\sigma_{\hat{C}_{dye}}^2 = \sigma_{\hat{\mu}_{t,\lambda}}^2 + \sigma_{\hat{\mu}_{t,\lambda_2}}^2.
\]

### 3 Materials and Methods

#### 3.1 Tissue Phantoms

As a simple tissue phantom we use 1.5% Agar gel in Aqua dest, with the addition of Intralipid (IL) to introduce scatter. We created samples having 2-D scattering properties by molding layered samples where the layers have a spatially varying thickness and different IL concentration. As the analyte, we used aluminum phthalocyanine tetrasulfonate chloride (AlPcS834, Porphyrin Products, Inc.), a PDT-related dye with one dominating absorption peak centered at 675 nm. The refractive index of the gel phantoms is \( n = 1.34 \).
2.5 by the spot size of the focused probing beam, as described in Transversal resolution and thus speckle size was determined An identical lens is used in front of the reference mirror in the coherence length is reduced to \( \lambda_c \). Sources are two pigtailed superluminescent diodes \( \text{SLDs} \) multiplexed OCT system used for acquiring OCT images. The Figure 1 shows a simplified schematic of the wavelength-\( \mu_2,675 \) and \( \mu_2,809 \), respectively. We measured the coherence lengths to be \( \lambda_c,5 \approx 18.1 \mu m \) for the 675-nm source and \( \lambda_c,5 \approx 14.2 \mu m \) for the 809-nm source. These coherence lengths correspond to spectral FWHM of 11 and 20 nm for the 675- and 809-nm sources, respectively, assuming Gaussian spectra. In a sample of group refractive index \( n_g \), the coherence length is reduced to \( \lambda_c = \lambda_c,5 / n_g \). Light from the two sources is multiplexed in a 2\( \times \)2 integrated fiber coupler and collimated before it is launched into the bulk Michelson interferometer. The interferometer is shot-noise limited and has a dynamic range of 90 dB. We used focus tracking to ensure that the focus of the probing beam overlaps with the coherence volume of the interferometer. The focusing lens was scanned along with the reference mirror using lens velocity \( v_r = v_r,5 / n_g \), where \( v_r \) is the velocity of the reference mirror. For all the experiments presented, the reference-mirror scanning velocity was \( v_r = 1 \text{ mm/s} \).

The light was focused into the sample by a focusing lens (Melles Griot 06GLC003) having focal length \( f = 14.5 \text{ mm} \). An identical lens is used in front of the reference mirror in order to match dispersion in the two interferometer arms. Transversal resolution and thus speckle size was determined by the spot size of the focused probing beam, as described in Sec. 2.2. The \( 1/e^2 \) beam diameter was around 5 mm, yielding a theoretical transversal resolution of approximately \( \lambda_t \approx 2.5 \mu m \). According to the lens manufacturer, the focusing lens has a focal shift of about 100 \( \mu m \) between the two probing wavelengths, resulting in a degrading of the transversal resolution at one or both wavelengths, depending on the overlap between the focus and the coherence area. Through experiments, we have found that a transversal displacement of 10 \( \mu m \) is sufficient for the speckle signal in two adjacent A scans to be decorrelated at both wavelengths.

### Table 1: Optical properties of 1.5% Agar gel and aluminium phthalocyanine dye.

<table>
<thead>
<tr>
<th>( F )</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_{1,675} ) / ( \mu_{1,809} )</td>
<td>1.5 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>( \varepsilon_{a,675} )</td>
<td>( \mu_{a,675} / C_{\text{dye}} )</td>
<td>44 ( a ) \text{ ml/mg mm}</td>
</tr>
<tr>
<td>( \varepsilon_{a,809} )</td>
<td>( \mu_{a,809} / C_{\text{dye}} )</td>
<td>&lt;10(^{-3}) ( a )</td>
</tr>
</tbody>
</table>

\(^a\) Typical value. Accurate value determined for each individual stock solution of dye.

4 Experimental Results

Experiments were performed on several sample objects of Agar gel prepared with different concentrations of IL and phthalocyanine dye. Homogenous samples with constant dye and IL concentrations, and one-dimensional (1-D) samples with IL concentration varying only in the \( z \) direction were used to investigate the influence of transversal and axial speckle averaging on the precision and resolution in estimating the depth-resolved optical properties. These measurements were also used to study the relation between field reflectivity and scattering coefficient. Two-dimensional cross-sectional images of three-dimensional (3-D) samples with IL concentration varying both in depth and in the plane normal to the optical axis of the probing light were also recorded. Functional images of optical properties and dye concentration were found from the structural OCT images recorded at two wavelengths. Results are presented in the following sections.

4.1 Determining Tissue-Phantom Parameters

To determine the dye concentration from the OCT images using Eq. (4), initial measurements were performed to find the necessary parameters for the phantoms. A commercial absorption spectrometer (Agilent 8452 UV-visible spectrophotometer) was used to determine the extinction coefficient of the dye at the two probing wavelengths. Measurements on Agar samples without dye, using the OCT setup, determined an empirical value for \( F \) for several IL concentrations. For this purpose, the scattering coefficient was determined, for both wavelengths, by linear regression on the logarithm of the OCT envelope, transversally averaged over a large area of the sample, and Eq. (3) was employed. For all IL concentrations used in the current experiments, the same value for \( F \), within the measurement uncertainty, was found. Experimentally determined values for \( F \) and \( \varepsilon_{a,\lambda} \) are summarized in Table 1. Note that the dye extinction coefficient was found to be more that three orders of magnitude larger at 675 than at 809 nm. In the further calculations, we therefore use \( \varepsilon_{a,809} = 0 \).
4.2 Speckle Noise in Estimation of the Attenuation Coefficient and Dye Concentration

To get a qualitative impression of the effect of transversal and axial speckle averaging on the estimated attenuation coefficient, we analyzed OCT images recorded from a homogenous Agar sample containing 0.15% IL. Figure 2(a) shows the logarithm of the image function recorded at 675 nm. The Agar samples were covered with a microscope glass slide, and the dip in the signal right below the glass-Agar reflection is due to a thin layer of water between the glass and the gel. To obtain an estimate of the depth-resolved attenuation coefficient we used Eq. (16). The image function was filtered in the axial direction by convolution with a filter function while no further filtering was done transversally. The axial filter function was chosen to be a Gaussian function truncated at $\sigma_w = \frac{h}{2}$. The Gaussian is a common filter function due to its lack of side-lobes in both position and spatial frequency. Truncating it introduces some side-lobes in the spatial-frequency plane, but provides strict control over which parts of the filtered data are affected by edge effects. As characteristic length of the filter function $L_z$ we used the full width at half maximum. Figure 2(b) shows the resulting depth-resolved attenuation coefficient for $m = 60$ and $L_z = 0.30$ mm (solid line) and $m = 30$ and $L_z = 0.15$ mm (dotted line) obtained from the data to the right of the vertical dashed line in Fig. 2(a). As expected from Eq. (22) we see that increasing $m$, and $L_z$ greatly improves the precision in the estimated attenuation coefficient. Note that as long as the backscattered signal is well above the noise level of the system, speckle noise will be the dominant source of noise in the image function. The noise sources defining the noise level of the system will thus not influence the analysis of the variance in the estimated attenuation coefficient.

To quantify the effect of transversal and axial speckle averaging on the precision in the estimated attenuation coefficient $\hat{\mu}_t$, the standard deviation of the estimated depth-resolved attenuation coefficient $\sigma_{\hat{\mu}_t}$, was found experimentally for several values of $m$ and $L_z$. As the axial filter function we used a Gaussian function. The solid lines in Fig. 3 are a contour plot of $\sigma_{\hat{\mu}_t}$ determined experimentally from measurements on an Agar sample containing 0.15% IL using the source at 675 nm. The two crosses indicate the parameters used in Fig. 2(b). To compare the experimental results with the theoretical predictions, we plotted the theoretical expression from Eq. (22) along with the experimental results. To obtain the good agreement shown in Fig. 3, the constant $K_t$ in Eq. (22) was multiplied by a factor 1.6. Thus, the deviation between the theoretical predicted and experimentally determined values for the variance in the estimated attenuation

![Fig. 2](image1.png)

![Fig. 3](image2.png)
coefficient was 26%. For the 809-nm source, the deviation was found to be 14%.

Next we estimated the depth-resolved attenuation coefficient at both probing wavelengths, and an estimate for the sample dye concentration was found using Eq. (23). Measurements were performed on homogenous Agar samples containing both IL and dye. We prepared samples having three different nominal dye concentrations, $C_{dye}=0.000 \text{ mg/ml}, 0.030 \text{ mg/ml},$ and $0.045 \text{ mg/ml},$ all having the same concentration of IL, $C_{IL}=0.15\%$. Figure 4(a) shows the average of the OCT envelope of $m=200$ transversally displaced and uncorrelated A scans recorded at 675 and 809 nm for Agar gel having $C_{dye}=0.030 \text{ mg/ml}$. The plots clearly show the difference in attenuation at the two wavelengths resulting in a steeper slope for the envelope recorded at 675 nm (black line) than at 809 nm (gray line).

The depth-resolved attenuation coefficient of the samples was determined using Eq. (16) for both wavelengths. Results for the 0.030-mg/ml sample are shown in Fig. 4(b). Again we used a truncated Gaussian function having an FWHM of $L_z=0.25 \text{ mm}$ as an axial filter function. The solid, vertical lines delimit the interval unaffected by edge effects in the filtering. Only the data to the right of the vertical dashed line in Fig. 4(a) were used for estimating the attenuation coefficients.

Using Eq. (23), an estimate of the depth-resolved dye concentration was obtained based on the two depth-resolved attenuation coefficient estimates. Estimated concentrations are plotted in Fig. 4(c) for typical measurements on Agar samples prepared using all three dye concentrations. The dotted horizontal lines show the nominal dye concentration determined from the amount of dye added to the samples during preparation. We see that in the interval where results are unaffected by edge effects, there is good agreement between the nominal and estimated dye concentration, with root mean square (rms) deviations within the interval 0.0033 to 0.0040 mg/ml for the three samples. As expected from Sec. 2.3, the standard deviation of the estimated dye concentration is independent of the value of the dye concentration with an average standard deviation for the three samples being $\langle \sigma_{\hat{C}} \rangle=0.0028 \text{ mg/ml}$, within the interval unaffected by edge effects. This is in good agreement with the theoretical value $\sigma_{\hat{C}}=0.0022 \text{ mg/ml}$ found from Eq. (24) using the current speckle-averaging and source parameters. The deviation between the experimental and theoretical value probably originates from the deviation found in the analysis of Fig. 3.

4.3 Spatial Resolution in Determining the Optical Properties

To study the effect of sample inhomogeneities on the estimation of optical properties, we prepared two-layered Agar samples having different IL concentrations but the same dye concentration in the two layers. Figure 5(a) shows the transversally averaged OCT envelope from a sample having a 1-mm layer where $C_{IL1}=0.05\%$ on top of a layer where $C_{IL2}=0.15\%$. The dye concentration in both layers is $C_{dye}=0.030 \text{ mg/ml}$. Note that there is a step in the level of the backscattered signal at the interface between the two layers due to the increased backscattering, and that the slope of the averaged envelope is steeper in the second than in the first layer. When differentiating a signal such as this, the difference

![Fig. 4](image-url)
Functional imaging of dye concentration...
Using Eq. (16) we obtain estimated images of the scattering coefficient at the two wavelengths. Results are shown in Figs. 8(a) and 8(b) for 675 and 809 nm, respectively. The estimated optical properties are obtained using $m = 1$ in the image function. The transversal displacement for each A scan is $\Delta x = 10 \mu m$, found experimentally to be large enough to ensure uncorrelated speckle noise in adjacent A scans for both wavelengths. The transversal and axial filter functions are chosen to be Gaussian functions truncated at $\pm 2\sigma$ and normalized to unit area. The characteristic lengths of the filter functions, the FWHM of the Gaussian functions, for the images in Fig. 8 are $L_z = L_t = 0.20 \text{ mm}$. According to Eq. (17), each pixel in the images is thus an average over approximately $N \times M = 270$ and $340$ independent speckle realizations for the images recorded at 675 and 809 nm, respectively. The interface between the two agar layers can be clearly seen in the images due to the step artifact discussed in Sec. 4.3. The difference in the attenuation coefficient in the two layers is evident in the images, and we can also see that the estimated attenuation coefficient has a constant mean value within each layer.

Finally, Fig. 8(c) shows functional OCT images of estimated dye concentration calculated from Figs. 8(a) and 8(b) using Eq. (23). Apart from the artifact arising from the interface between the two layers, the image shows a fairly constant
Functional imaging of dye concentration...

The estimated dye concentration averaged over the two sample layers, excluding edge and interface regions, is \( \langle \hat{C}_{\text{dye}} \rangle = 0.028 \text{ mg/ml} \) with a standard deviation of \( \sigma_{\hat{C}} = 0.010 \text{ mg/ml} \), compared to nominal value \( C_{\text{dye}} = 0.030 \text{ mg/ml} \).

The functional dye-concentration image shows that even though the experimental results are in good agreement with the expected value within the two layers, the step in reflectivity at the layer interface influences a significant part of the image. For more realistic tissue phantoms and, eventually, for measurements on tissue, it might be expected that local variations in reflectivity may corrupt the dye-concentration images. Figure 9(a) shows the transversally averaged OCT envelope for the data presented in Figs. 7 and 8. In Figs. 9(b) and 9(c) the attenuation coefficient and the dye concentration are calculated following the same procedure as used in Sec. 4.2. Figure 9(c) shows that even though the scattering of the sample varies transversally, the resulting calculated depth-resolved dye concentration is in good agreement with the theoretical value as a function of depth.

![Figure 9](image)

**Figure 9** (a) Logarithm of the image function \( P_{m,\lambda} \), \( m = 250 \), obtained by averaging all A scans of Figs. 7(a) (black line) and 7(b) (gray line); (b) estimated attenuation coefficient at the two wavelengths based on the image functions in (a), \( L_z = 0.20 \text{ mm} \); and (c) estimated dye concentration calculated from the estimated attenuation coefficients in (b). We observe that even though the scattering of the sample varies transversally along the sample, the resulting calculated depth-resolved dye concentration is in good agreement with the theoretical value as a function of depth.

dye concentration. The estimated dye concentration averaged over the two sample layers, excluding edge and interface regions, is \( \langle \hat{C}_{\text{dye}} \rangle = 0.028 \text{ mg/ml} \) with a standard deviation of \( \sigma_{\hat{C}} = 0.010 \text{ mg/ml} \), compared to nominal value \( C_{\text{dye}} = 0.030 \text{ mg/ml} \).

The functional dye-concentration image shows that even though the experimental results are in good agreement with the expected value within the two layers, the step in reflectivity at the layer interface influences a significant part of the image. For more realistic tissue phantoms and, eventually, for measurements on tissue, it might be expected that local variations in reflectivity may corrupt the dye-concentration images. Figure 9(a) shows the transversally averaged OCT envelope for the data presented in Figs. 7 and 8. In Figs. 9(b) and 9(c) the attenuation coefficient and the dye concentration are calculated following the same procedure as used in Sec. 4.2. Figure 9(c) shows that even though the scattering of the sample varies transversally, the resulting calculated depth-resolved dye concentration is in good agreement with the nominal value. The mean value of the estimated dye concentration is \( \langle \hat{C}_{\text{dye}} \rangle = 0.030 \text{ mg/ml} \) and the standard deviation is \( \sigma_{\hat{C}} = 0.005 \text{ mg/ml} \). This is an indication that even for samples having large variations in scattering properties, it may be possible to obtain reliable estimates of the dye concentration at the expense of transversal resolution, if only the scattering properties vary within the limitation of the model as it is presented in Sec. 2.1.

5 Discussion

The presented results show that it is possible to obtain functional images of the concentration of an analyte in a scattering Agar-IL tissue phantom based on OCT measurements at two wavelengths. The following is a discussion of factors determining the precision in the estimated dye concentration and some of the challenges in the design of a dual-wavelength concentration-imaging OCT system.

In this paper, speckle averaging is limited to spatial averaging, and we do not discuss speckle-noise reduction through polarization diversity,\(^2\) frequency compounding,\(^2\) or advanced image processing.\(^3\) As a measure of precision in the estimated attenuation coefficients and dye concentration, we use the variance of the estimated parameters. According to Eq. (22), the variance of \( \hat{\mu}_r \) is proportional to the source coherence length \( L \), and the transversal resolution \( l_t \), inversely proportional to the width of the transversal filter function \( L_z \), and inversely proportional to the third power of the width of the axial filter function \( L_d \). Thus, there is a trade-off between spatial resolution and precision in the estimated attenuation coefficient. An increase of the axial filter width gives a larger improvement in precision compared to the same increase in transversal filter width. Imaging with a shorter coherence length, or a larger numerical aperture, will reduce speckle size in the axial and transversal directions, respectively. For a given spatial resolution, determined by the filter lengths, the precision in the estimated attenuation coefficient will thus be improved because the number of independent speckles within the filter lengths increases. A shorter coherence length can be obtained using a broader bandwidth source as long as dispersion in the sample under study is small enough to avoid problems due to dispersion broadening.

Few studies have been done on the precision of estimated attenuation coefficients from tissue. Schmitt et al.\(^4\) report values of the attenuation coefficient of human tissue from different sites on the body determined with an average standard deviation of \( \sigma_{\mu} = 0.5 \text{ mm}^{-1} \). The attenuation coefficient at the different sites varied in the range 2 to 5 mm\(^{-1}\). This result was obtained using linear regression over \( L_d = 0.50 \text{ mm} \) with no transversal averaging of the OCT envelope, and is of the same order of magnitude as \( \sigma_{\mu} \) predicted by the expression in Eq. (22). Kholodnykh et al.\(^5\) state that they have determined the scattering coefficient of tissue with a precision of 0.8%, corresponding to a standard deviation of \( \sim 0.05 \text{ mm}^{-1} \) for typical values of the scattering coefficient of tissue. To achieve this precision \( 5 \times 10^4 \) A scans were averaged over a transversal area of 3.4×4.3 mm, and the attenuation coefficient was found as the slope of the logarithm of the averaged data using linear regression over a depth of 0.25 mm. For these averaging parameters, and a reported coherence length of \( l_c = 15 \text{ mm} \), Eq. (22) predicts a theoretical standard deviation of \( \sim 0.004 \text{ mm}^{-1} \), thus an order of magnitude better than estimated by Kholodnykh et al.

Typical values for the attenuation coefficient of human tissues are \( >1 \text{ mm}^{-1} \). Equation (22) shows that for a coherence length in the sample of 15 mm and a transversal resolution of 10 mm, a variance in the estimated attenuation coefficient of
\( \sigma_{\mu} = 0.01 \text{ mm}^{-1} \) is obtained for \( L_z = L_t = 1.03 \text{ mm} \). Reducing the filter lengths to \( L_z = L_t = 0.46 \text{ mm} \) results in a variance of \( \sigma_{\mu} = 0.05 \text{ mm}^{-1} \). These results are obtained on samples having an attenuation coefficient of 1 to 2.5 \( \text{mm}^{-1} \), just within realistic values for human tissues. For samples having a higher attenuation coefficient, the object depth for which we have a useful signal will be reduced. This limits the maximum filter length and thereby the precision in the attenuation-coefficient estimator.

Equations (22) and (24) show that the precision in the estimated dye concentration is independent of the dye concentration value. This is confirmed by experimental results presented in Fig. 4(c). For a given set of speckle-averaging parameters, the resulting precision represents a fundamental limit on detectability of low dye concentrations. Equation (24) expresses how the variance in \( \hat{C}_{\text{dye}} \) depends on the variance in \( \mu_1 \). The choice of probing wavelengths and thus the values of \( F, \varepsilon_{a, \lambda}, \) and \( \varepsilon_{e, \lambda} \), together with \( \sigma_{\mu, \lambda}^2 \), determine the precision of the estimated dye concentration. According to Eq. (22) the variance in \( \mu_1 \) is determined by the speckle-averaging parameters, transversal resolution, and the source coherence length. Assuming that we can choose source parameters giving \( \sigma_{\mu, \lambda}^2 = \sigma_{\mu, \lambda}^2 = \sigma_1^2 \), for any \( F \), and introducing the parameter \( E = \varepsilon_{a, \lambda}/\varepsilon_{e, \lambda} \), Eq. (24) simplifies to \( \sigma_{\mu, \lambda}^2 = K(1 + F^2)/(1 - EF)^2 \), where \( K = \sigma_1^2/\varepsilon_{a, \lambda}^2 \). When imaging a dye having a narrow absorption band centered at \( \lambda_1 \) and \( E = 0 \), the second probing wavelength \( \lambda_2 \) should be chosen to yield \( F < 1 \), i.e., the scattering at \( \lambda_2 \) should be larger than at \( \lambda_1 \). This will give an improved precision in \( \sigma_{\mu, \lambda}^2 \) compared to using a wavelength yielding \( F > 1 \). For the sources used in this paper, \( F > 1 \). Generally the best precision in \( \hat{C}_{\text{dye}} \) is obtained when both \( F \) and \( E \) are \( \ll 1 \). An additional requirement is that the total attenuation at both wavelengths must be weak enough to give a sufficiently large penetration depth for good determination of the slope of the OCT envelope.

The presented method is limited to samples with relatively homogeneous scattering. The whole sample is represented by the same \( F \), and this \( F \) value is assumed not to change with concentration of the analyte under study. Faber et al. showed that blood oxygenation changes the scattering properties of whole blood.25 An effect of this kind will complicate the analysis. In addition, the dye must be the only absorbing component in the sample. This will not generally be the case for tissue where several other chromophores (e.g., hemoglobin and melanin) may contribute to the absorption, thus complicating the selection of suitable wavelengths.

A challenge when using two or more probing wavelengths in the interferometer lies in the choice of optical components. Unless the focusing lens is completely corrected for chromatic aberrations at all probing wavelengths, differences in focal length will result in degraded transversal resolution at all but one of the wavelengths, since the coherence area can not overlap with the focus area for all wavelengths simultaneously. Dispersive samples will complicate the situation further since the coherence area will be located at different geometrical depths for different wavelengths.

Note that Eq. (16) includes an additional implicit approximation in assuming that all the light recorded at depth \( z \) has traveled the same path length in the sample. This approximation will be increasingly inaccurate as the numerical aperture (NA) for the system increases and light passing through the outer part of the aperture may have traveled a significantly longer distance in the object than light traveling close to the optic axis. For large-NA systems, this will give an incorrect estimate of the attenuation coefficient if not corrected for.

Focus tracking is necessary to extract quantitative information about the attenuation coefficient of a sample from OCT images. An alternative to focus tracking is correcting the recorded data for the effect of the confocal function.34,34 For samples with relatively high scattering, focus tracking is the better alternative since it gives a larger penetration depth, enabling more axial filtering and thus better precision in the determined optical properties. Finally, a large dynamic measurement range is required for high penetration depth.

This work was carried out using a dual-source interferometer with wavelengths tuned to the analyte under study. An OCT system using an ultrabroadband source would enable measurements at a wide range of wavelength bands, providing a flexible solution for imaging of analytes with different absorption properties.

6 Conclusion

This paper demonstrated quantitative functional imaging of the concentration of an absorbing analyte in a scattering tissue phantom by means of spectroscopic OCT. We analyzed the accuracy of the dye-concentration estimate and discussed sources of error that apply to the measurements. Good agreement was demonstrated between measured and predicted variances in the estimated attenuation coefficients and dye concentration. We presented guidelines for spectroscopic OCT systems for concentration imaging and discussed some of the possible obstacles for the application of the method to more realistic phantoms and tissue. Tissue is a complicated sample and it is difficult to predict what will be the dominating obstacles when using this method on live tissue. Nevertheless, this paper demonstrated that the goal of quantitative depth-resolved measurements of dye concentration is, in principle, possible to achieve provided that a sufficient model for the optical properties of the tissue is found.

Acknowledgments

This work was supported by the Research Council of Norway. The authors wish to thank Prof. Ole J. Løskberg and Prof. Hans M. Pedersen for helpful discussions on instrumentation and speckle averaging, respectively.

References