Separation of absorption and scattering properties of turbid media using relative spectrally resolved cw radiance measurements

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Abstract: We present a new method for extracting the effective attenuation coefficient and the diffusion coefficient from relative spectrally resolved cw radiance measurements using the diffusion approximation. The method is validated on both simulated and experimental radiance data sets using Intralipid-1% as a test platform. The effective attenuation coefficient is determined from a simple algebraic expression constructed from a ratio of two radiance measurements at two different source–detector separations and the same 90° angle. The diffusion coefficient is determined from another ratio constructed from two radiance measurements at two angles (0° and 180°) and the same source–detector separation. The conditions of the validity of the method as well as possible practical applications are discussed.

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1. Introduction

Extraction of optical parameters of biological tissues is of great interest for biomedical optics for tissue diagnostics, treatment planning or monitoring [1–3]. For example, the in vivo determination of optical properties of cancerous tissue is needed to optimize the light dose distribution during photodynamic therapy [4–6]. When light propagates in turbid media, both absorption and scattering by tissue contribute to the total attenuation of light, characterized by two optical parameters, the effective attenuation coefficient ($\mu_{\text{eff}}(\lambda)$) and the diffusion coefficient ($D(\lambda)$) when the diffusion approximation is valid [7]. A detailed coverage of various aspects of light interaction with turbid media is given in several excellent books on this subject [8–10]. The effective attenuation coefficient quantifies the combined effect due to absorption and scattering and is of great value by itself. Both $\mu_{\text{eff}}(\lambda)$ and $D(\lambda)$ can be determined by fitting measured optical data to a diffusion model. Further, the absorption coefficient ($\mu_a(\lambda)$) and the reduced scattering coefficient ($\mu'_s(\lambda)$) can be determined using the following relations: $D(\lambda) = 1/[3\mu'_s(\lambda) + \mu_a(\lambda)]$ and $\mu_{\text{eff}}(\lambda) = \sqrt{\mu'_s(\lambda) / D(\lambda)}$ [8–11]. Typically, all techniques can be classified based on a type of light sources used to interrogate tissue and are divided into three categories: continuous wave (cw), intensity modulated and time resolved [2,7,12] (see also Chap. 8 in Ref. [8]). Figure 1 shows a typical schematic representation of responses of a turbid medium to external stimuli used in various

![Fig. 1. Schematic representation of responses of the turbid media to external stimuli used in various measurement approaches.](image-url)
measurement approaches. An external stimulus is shown on a left from the block representing a turbid medium, while a medium response produces a modified signal shown on a right from the block. Since all considered measurement approaches involve some sort of intensity measurements, Y-axis is labeled as “intensity”. X-axes are linked to a domain where measurements are performed. The techniques are listed in the order of increased information content that can be extracted from a single source–detector measurement pair (i.e., optode).

The continuous wave (or intensity) techniques are usually associated with fluence-type measurements where isotropic detectors collect photons from 4π solid angle. An external stimulus is usually a constant intensity laser or a multispectral light source. Tissue response results in attenuation of the input signal. One of first measurements of spatially resolved steady-state reflectance for determination of $\mu_a$ and $\mu_s'\text{ of the tissue}$ was reported by Reynolds et al. [13]. Since then, reflectance has been used frequently for determination of optical properties of turbid media [14–21]. Performing relative fluence measurements at multiple source–detector separations enables the determination of $\mu_{a}(\lambda)$ from a value of the slope [22,23]. A variation of multi-distance fluence measurements, called the added absorber technique allows obtaining both $\mu_a$ and $\mu_s'$ by changing the absorption coefficient by small known quantities [24,25]. Otherwise, to obtain $D(\lambda)$ absolute fluence measurements with complex calibrations are required [26–28]. Such calibrations can be difficult to implement in vivo.

Another technique that belongs to the same cw family but used less often is radiance, whereby a detector with a well-defined angular aperture is rotated through 360° enabling the determination of the angular distribution of photons at a selected point in tissue. The interest in the angular distribution of light has been around since the early days of biomedical optics [3,29]. Radiance measurements have been used for studies of liquid phantoms and biological tissues [30–41] albeit being overshadowed by more popular fluence measurements. Scattering interferes with a spatial confinement of light beam spreading photons both in the spatial and angular domains [8,32]. Light from an interstitial source which is angularly confined loses intensity and broadens while propagating in tissue due to the diffuse nature of light. The specific effect of different media such as air, water and Intralipid-1% on the angular broadening of experimentally measured radiance is presented in Ref. [39]. While fluence and radiance both measure cw light intensity, angular-resolved measurements of radiance provide added value increasing the information content available to recover the optical properties of tissue.

Intensity modulated techniques rely on exploring the diffusive wave in tissue that is produced by modulating the light source with a frequency in a range of 100 MHz – 1 GHz [2,42–48]. In response to an external intensity modulated stimulus, the medium attenuates the signal and introduces a phase shift relative to the input signal. Obtaining the change in modulation and the phase allows for both $\mu_a(\lambda)$ and $\mu_s'(\lambda)$ determination. In time resolved techniques a short pulse of light (<100 ps) is launched to the medium at a selected point and time. As it propagates through tissue, the pulse undergoes attenuation and broadening that can be used to extract $\mu_a(\lambda)$ and $\mu_s'(\lambda)$ [49–53]. It was found that pulse shape (rather than the fluence rate levels) analysis makes the technique insensitive to local bleeding at fiber tips [52,53]. One can also notice certain parallels between transformations of initially confined light excitation in the angular and time domains.

In order for techniques to be used in clinical applications in vivo they should ideally be minimally invasive (limited fiber translations), provide complete tissue characterization without requiring complex absolute calibrations, enable the detection and characterization of tissue inhomogeneities and be cost effective. Frequency-domain and time-domain techniques enable the extraction of optical absorption and scattering from a single optode pair (i.e., minimally invasive) without absolute calibrations, but they require more complex and costly equipment than cw-based methods.
The goal of this article is to demonstrate that our cw-based radiance approach doesn’t have limitations inherent to cw-based fluence techniques, and still allows for accurate determination of both $\mu_a(\lambda)$ and $\mu'_a(\lambda)$ from relative measurements requiring only a rotation and a single translation in tissue with a potential of completely eliminating the translation. It may bring cw-based radiance closer to frequency and time domain techniques in terms of being able to provide a complete tissue characterization from a single optode measurement.

2. Methods and materials

A detailed description of the experimental setup for radiance measurements has been published elsewhere [39,41]. Therefore, only a brief description is given here. A schematic of the experimental setup is shown in Fig. 2. A phantom Lucite box (with blackened 18-cm walls) was filled with Intralipid-1% solution and accommodated a fiber with a 800-μm spherical diffuser (connected to a white light source) and a 600-μm side firing fiber (the radiance detector). A size of the box insured an infinite medium geometry. The side firing fiber had a well-defined angular acceptance window of ~10 degrees in water. Both fibers were threaded through ~1.1-mm stainless steel tubes for mechanical stability. Source–detector separation was varied from 6.5 mm to 30.5 mm in various measurements. The side firing fiber was mounted on a computer-controlled rotation stage. The radiance profiles were acquired by rotating the side firing fiber over a 360 degree range with a 2 degree step. The side firing fiber was connected to a computer-controlled USB 4000 spectrometer (Ocean Optics) that collected spectra at every angular step.

![Fig. 2. A schematic of the experimental setup for radiance measurements.](image)

A method of extracting of optical properties of the turbid medium from radiance measurements was introduced earlier [41]. Briefly, we evaluated the ability to extract $\mu_{\text{eff}}(\lambda)$ and $D(\lambda)$ for Intralipid-1% (450–950 nm range) using the diffusion approximation from both experimental and simulated distance-dependent radiance data sets. Simulated radiance data sets have been obtained using an analytical solution of the RTE (radiative transfer equation) for the radiance caused by an isotropic light source placed inside an infinitely extended anisotropic scattering medium [54]. Radiance was expressed as a function of optical properties of Intralipid-1% ($\mu_a(\lambda)$, $\mu'_a(\lambda)$ and anisotropy factor, $g(\lambda)$) that were obtained directly from (non-radiance) collimated transmission and spatially resolved reflectance measurements (called basic characterization measurements). Thus, known optical properties of Intralipid were input parameters for simulated radiance data sets. Then both simulated and experimentally obtained radiance data sets were fit to a formula of relative radiance derived from the diffusion-based expression for radiance to recover $\mu_{\text{eff}}(\lambda)$ and $D(\lambda)$. Using multiple distance measurements within a 6.5–30.5 mm range (with detector facing the source, i.e. 0° angle) and normalizing the radiance data to the value obtained at the shortest source–detector separation (6.5 mm, that is approximately 6 optical penetration depths away from the light source), we demonstrated a successful recovery of $\mu_{\text{eff}}(\lambda)$ but failed to recover $D(\lambda)$. This may be a result of the use of relative radiance, in that it didn’t provide enough resolving power to
extract both parameters from the fit data. Recovery of optical parameters from relative radiance built in this way likely suffered from non-uniqueness due to optical similarity. The current article proposes a better solution that recovers both parameters.

A starting point for the current approach is also the expression for the radiance, \( I \) under the diffusion approximation:

\[
I(r, \theta, \lambda) = \frac{P_0}{(4\pi)^2 \cdot D(\lambda)} \left[ 1 + 3 \left( \frac{D(\lambda)}{r} + \mu_{\text{eff}}(\lambda) \cdot D(\lambda) \right) \cdot \cos \theta \right] \exp\left(\frac{-\mu_{\text{eff}}(\lambda) \cdot r}{r}\right) \tag{1}
\]

where \( r \) is a distance between the source and the detector, \( \theta \) is the angle between the direction of propagation and the direction of scattering, \( \lambda \) is a wavelength, \( P_0 \) is the source power. In order to exclude \( P_0 \) and \( D(\lambda) \) and obtain an expression that depends solely on \( \mu_{\text{eff}}(\lambda) \), a ratio of two radiance values measured at two different distances, \( r \) but the same 90° angle was constructed:

\[
\frac{I(r,90^\circ,\lambda)}{I(r_0,90^\circ,\lambda)} = \exp(-\mu_{\text{eff}}(\lambda) \cdot r) \cdot \exp(\mu_{\text{eff}}(\lambda) \cdot r_0 / r), \tag{2}
\]

where \( r_0 \) was chosen to correspond to the shortest source–detector separation. Equation (2) can be arranged to solve for \( \mu_{\text{eff}}(\lambda) \) as follows:

\[
\mu_{\text{eff}}(\lambda) = \ln(\frac{I(r,90^\circ,\lambda) \cdot r}{I(r_0,90^\circ,\lambda) \cdot r_0}) / (r_0 - r). \tag{3}
\]

To determine \( D(\lambda) \) another ratio was constructed using two different angles, 180° and 0° and the same distance, \( r \):

\[
\frac{I(r,180^\circ,\lambda)}{I(r,0^\circ,\lambda)} = \frac{-3 \cdot D(\lambda) + r \cdot (1 - 3 \cdot \mu_{\text{eff}}(\lambda) \cdot D(\lambda))}{3 \cdot D(\lambda) + r \cdot (1 + 3 \cdot \mu_{\text{eff}}(\lambda) \cdot D(\lambda))}. \tag{4}
\]

With knowledge of \( \mu_{\text{eff}}(\lambda) \) from Eq. (3), Eq. (4) can be solved for \( D(\lambda) \) as follows:

\[
D(\lambda) = \frac{1 - I(r,180^\circ,\lambda) / I(r,0^\circ,\lambda)}{3(\mu_{\text{eff}}(\lambda) + 1 / r)(1 + I(r,180^\circ,\lambda) / I(r,0^\circ,\lambda))}. \tag{5}
\]

The advantage of this approach (Eqs. (3) and (5)) is that data fitting, multiple and absolute measurements are not required, rather both \( \mu_{\text{eff}}(\lambda) \) and \( D(\lambda) \) can be determined directly from simple algebraic expressions that contain only measured quantities.

3. Results and discussion

This approach was tested first with simulated radiance using \( P_\infty \) approximation as described by Grabtchak et al. [41]. Results of applying Eq. (3) to different distance-pairs are shown in Fig. 3(a) along with the actual values of \( \mu_{\text{eff}}(\lambda) \) measured independently [41]. All distance-pair data produced excellent agreement with actual \( \mu_{\text{eff}}(\lambda) \) experimental values. Figure 3(b) shows results of applying Eq. (5) to determine \( D(\lambda) \) at different distances. Actual values of \( D(\lambda) \) are also shown in the plot. Again, the agreement between the extracted and actual values is excellent. The plot also shows that spectrally resolved radiance obtained at any distance produces identical results. This represents a strong contrast to the inability of retrieving correct values of \( D(\lambda) \) from simulated results using the earlier approach from our group [41].

Results of recovery of \( \mu_{\text{eff}}(\lambda) \) and \( D(\lambda) \) from experimental radiance using previously published experimental data from Ref. [41] are shown in Fig. 4(a,b). The recovery of \( \mu_{\text{eff}}(\lambda) \), in Fig. 4(a), is remarkable given that all experimental values of radiance and distance contain up to 4% random error. The plot indicates that any distance pair within the experimental range of 6.5–30.5 mm can be used to recover \( \mu_{\text{eff}}(\lambda) \) within ±2%. The recovery of \( D(\lambda) \) from experimentally measured radiance sets is presented in Fig. 4(b) where up to 40% error is observed for short wavelengths with the error decreasing nearly to zero with increasing wavelength to 900 nm. It is important to note that the expression for \( D(\lambda) \) includes
Fig. 3. A comparison of optical parameters extracted from simulated data (lines) with actual parameters obtained from basic characterization experiments (symbols): a) extracted $\mu_{\text{eff}}(\lambda)$ based on Eq. (3) for different pairs of source–detector separations, b) extracted $D(\lambda)$ based on Eq. (5) for different source–detector separations.

A ratio of the backscattered radiance to the forward scattered radiance. The discrepancy between known and radiance predicted $D(\lambda)$ is likely due to the radiance sensor design including a finite thickness of the fiber and tube, secondary rays that don’t undergo a total internal reflection in the fiber tip, and fiber material as was shown in Ref. 41. All these factors affect the ratio of the backscattered to forward scattered radiance. To illustrate this, the radiance at two wavelengths 550 nm and 850 nm was simulated using an analytical solution of the RTE and known optical properties of Intralipid-1% [54]. The difference between the measured and simulated radiance was 8% and 5% for 550 nm and 850 nm, respectively. By increasing the input experimental radiance by 8% (550 nm) and 5% (850 nm) the recovered $D(\lambda)$ agreed well with known values at these two wavelengths (shown as hollow green and red circles in Fig. 4). One can notice an overall spread in $D(\lambda)$ values obtained from different distance measurements that reflects random experimental errors. After accounting for a systematic error in the backscattered radiance, the residual error for $D(\lambda)$ is ±4%. The next step is to optimize the radiance sensor design to better align with the mathematical framework.

Fig. 4. A comparison of optical parameters extracted from experimental radiance data (lines) with actual parameters obtained from basic characterization experiments (symbols): (a) extracted $\mu_{\text{eff}}(\lambda)$ based on Eq. (3) for different pairs of source–detector separations, (b) extracted $D(\lambda)$ based on Eq. (5) for different source–detector separations.
for $D(\lambda)$ recovery. In practical terms, the fiber thickness can be reduced but this comes with increased risk of breakage. One way to potentially eliminate secondary rays in the fiber tip is by introducing a protective heat-shrink white polymer cap with a window that blocks all secondary beams. This is under investigation and will help to inform an approach to correct for these experimental conditions.

This demonstrates the potential of this radiance approach to recover both optical parameters of the turbid medium with a few-percent error provided that the finite detector size and geometry are taken into account. In addition, there is no need to perform more than two distance dependent measurements and the distances can be separated by any values. It may be beneficial to perform measurements close enough to the source that would minimize noise which becomes more noticeable at higher source–detector separations above 850 nm as seen in Fig. 4(b).

Once $\mu_{\text{eff}}(\lambda)$ and $D(\lambda)$ have been obtained, $\mu_a(\lambda)$ and $\mu'_a(\lambda)$ can be determined from them using two relations listed in the introduction part as follows: $\mu_a(\lambda) = \mu_{\text{eff}}^2(\lambda) \cdot D(\lambda)$, $\mu'_a(\lambda) = (1 - 3 \cdot \mu_{\text{eff}}^2(\lambda) \cdot D^2(\lambda)) / 3 \cdot D(\lambda)$. Figures 5(a) and 5(b) demonstrate the spectral behavior of $\mu_a(\lambda)$ and $\mu'_a(\lambda)$ extracted from values of $D(\lambda)$ and $\mu_{\text{eff}}(\lambda)$ obtained from the radiance experiments at 12.5 mm source–detector separation with no correction applied. For comparison, the same parameters obtained from separate (non-radiance) basic characterization measurements are shown with symbols. One can see that the systematic error observed in $D(\lambda)$ translates to almost equivalent errors in both $\mu_a(\lambda)$ and $\mu'_a(\lambda)$. However, when experimental values of radiance data are corrected for backscatter, the error doesn’t exceed the value of a random error in $D(\lambda)$ (i.e., 4%).

We’d like to discuss the origin of a successful application of the diffusion approximation for radiance (Eq. (1)) even though the discrepancies for the angular radiance obtained from $P_1$ and $P_3$ on one hand and $P_\infty$ and Monte Carlo simulations on the other hand are well known and documented [11,36,54]. In order for the diffusion approximation to be valid two conditions must be fulfilled: $\mu_a \ll \mu'_a$ and $\mu'_a \cdot r >> 1$. A simple check of the first condition using our published data for Intralipid-1% [41] indicates that even in the range of high water absorption, the first condition is satisfied at $\lambda = 840$ nm: $\mu_a = 3 \times 10^{-3}$ mm$^{-1}$, $\mu'_a = 0.88$ mm$^{-1}$. The second condition fails at short and even medium distances used in the experiments giving
5.7 \gg 1 \text{ at } 6.5 \text{ mm and } 8 \gg 1 \text{ at } 10 \text{ mm which are not quite correct. Therefore, a discrepancy between the diffusion and } P_\infty \text{ radiance profiles is expected, and Fig. 6 demonstrates it for a 10 mm source–detector separation.}

Other wavelengths produce somewhat similar results. Note that both profiles in Fig. 6 are normalized to the value of the forward scattered radiance at 0° that hides the existing discrepancy in absolute values but still shows a different shape. Another important observation is that the ratios of the backscattered to the forward scattered radiance have the same value for both approximations. Since Eq. (4) was developed around this ratio, it explains why the use of the diffusion-based Eq. (1) produced accurate results. It becomes also evident that as long as any ratios formed from Eq. (1) involve the same angle (i.e., 90° and two distances) or probe the angular extremes of radiance (i.e., 0° and 180° and a single distance), the exact shape of the angular dependent radiance is not considered in the calculations. For example, our attempt to replicate the results forming a ratio between radiance values at 90° and 0° failed when testing with simulated data. Indeed, this ratio relies on a certain intermediate value of the angular radiance that has to be correct in order to produce valid results. Therefore, the approach described here can be applied beyond the formal conditions for the diffusion approximation. The exact range for the validity of this approach has to be tested with extensive modeling with } P_\infty \text{ approximation or Monte Carlo simulations. However, comparing normalized backscattered radiance for the diffusion approximation and Monte Carlo simulation using existing published data indicated that the condition of } \mu_s \ll \mu'_s \text{ is more important than } \mu'_s \cdot r \gg 1 \text{ in order for the approach to work. For example, when a medium has optical properties not satisfying the first condition, } \mu'_s / \mu_s = 5.7 \text{ ( } \mu'_s = 1 \text{ mm}^{-1}, \mu_s = 0.175 \text{ mm}^{-1} \text{) [36] measurements close to the source } (r = 5 \text{ mm}) \text{ and far from the source } (r = 13 \text{ mm}) \text{ produce, correspondingly 21% and 7% mismatch between normalized backscattered radiance values under two approximations. Treating this mismatch as errors in the backscattered radiance indicates that the error in the recovered } D(\lambda) \text{ would exceed 22%. However, the expression used to recover } \mu_g(\lambda) \text{ can still produce accurate values because the value of the backscattered radiance is not used in this calculation.}

We would like to put current findings in a perspective of a potential applicability to prostate measurements. Optical properties of prostate show a wide range of values indicating intra-organ and inter-patient variability. Still, using some reported optical properties we estimated the ratio } \mu'_s / \mu_s \text{ to produce values from 10 to 20 at NIR wavelengths [52]. This is a

![Fig. 6. Normalized to maximum at 0° angular radiance from analytical solution of RTE (solid red line) and from diffusion approximation (dashed blue line) for 850 nm and 10-mm source-detector separation.](image)
range where the first condition of the diffusion approximation for radiance is valid therefore we expect the demonstrated approach to retain its utility when applied to prostate.

As oppose to Intralipid, real tissues are more complex exhibiting various degrees of heterogeneity. Due to multiple scattering, radiance detects photons that travel through different regions in a sample. Thus, radiance measurements produce some averaged properties of the medium. Changes in these properties as a result of a diseased tissue state (e.g., cancer) or due to treatment can also be measured. Bleeding around interstitially inserted detector poses the same problem for any interstitial techniques, fluence or radiance. Blood around the detector would introduce unwanted increased optical absorption thus filtering the measured radiance signal coming from deeper tissues. When radiance measurements are performed on the prostate in a minimally invasive way (for example, illumination via rectum and detection via urethra), there should be no bleeding. According to the proposed method, this single point measurement produces enough data to determine the effective attenuation coefficient of the prostate. To determine the diffusion coefficient, only one additional interstitial point measurement is required, and this measurement may result in bleeding indeed. However, a need to puncture the prostate only once minimizes the degree of invasiveness in our opinion.

In order to eliminate a translation in tissue and extract all optical properties from angular measurements only, the need for the correct description of the angular radiance limits the use of the diffusion approximation. If a closed formed expression for radiance can be generated from Monte Carlo simulations or $P_\infty$ approximation, that would permit a separation of variables ($P_0$, $\mu'$, $\mu_s$, $g$), a typical radiance data set can provide a large number (up to 180 when taking data every $2^\circ$ in $0^\circ$–$360^\circ$ range) of independent equations to find the unknowns. Otherwise, all angular radiance data sets would increase a robustness of parameter recovery from simultaneous multiple curve fittings similar to the one used in a multi-spectral approach for DOT [2].

4. Conclusion

We have introduced a new approach that can be used to recover both $\mu_{\text{eff}}(\lambda)$ and $D(\lambda)$ of a turbid medium from relative spectrally resolved radiance measurements using the expression from the diffusion approximation. The approach requires probe rotation and only one translation in the medium. The explicit form of the angular dependence of the radiance is not involved in calculations but the accurate value of the ratio of the backscattered to the forward scattered radiance is required. It has been shown that when $\mu_s \ll \mu'_s$ both the diffusion approximation and more accurate descriptions like Monte Carlo or $P_\infty$ approximation produce the same value for the ratio that ensures a success of the approach even when the second condition for the diffusion approximation, $\mu'_s \cdot r >> 1$ is not valid.

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