Radiance detection of non-scattering inclusions in turbid media

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Abstract: Detection of non-scattering domains (voids) is an area of active research in biomedical optics. To avoid complexities of image reconstruction algorithms and requirements of a priori knowledge of void locations inherent to diffuse optical tomography (DOT), it would be useful to establish specific experimental signatures of voids that would help identify and detect them by other means. To address this, we present a radiance-based spectro-angular mapping approach that identifies void locations in the angular domain and establishes their spectral features. Using water-filled capillaries in scattering Intralipid as a test platform, we demonstrate perturbations in the directional photon density distribution produced by individual voids.

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1. Introduction

Propagation of light in turbid media containing non-diffuse (void) regions is a topic of considerable interest in biomedical optics [1–8]. The attention was triggered by application of diffuse optical tomography (DOT) for imaging the neonatal head that contains cavities filled with nearly transparent cerebrospinal fluid (CSF). These regions exhibit non-scattering (and possibly absorbing) properties and present difficulties for accurate diffuse light modeling when reconstructing DOT images containing CSF regions [7,8]. In fact, a priori knowledge of the locations of the non-scattering regions may be required for a successful application of a radiosity-diffusion model that can lead to an improved quality of the reconstructed image [3]. Thus, detection and identification of scattering-free voids can be considered in a more general context of detecting localized inclusions in turbid media and becomes an independent important subject for comprehensive investigation. For example, it is highly desired to identify specific signatures of simple void regions that would help detecting their presence (and possibly identify the composition) in the scattering background. In order to achieve a straightforward interpretation of experimental data and avoid complex reconstruction algorithms inherent to fluence-based multiple source-detector configurations, alternative experimental techniques can be explored. Another motivation for the current work was brought by our earlier study of localized inclusions based on colloidal gold in Intralipid [9]. In particular, it was noticed that the presence of water in the colloidal gold inclusion may be responsible for reduced detectability of the absorbing target at certain detection geometries. Photons interact differently with a transparent background liquid and with particles suspended in it, thus leading to mixed effects. Therefore, establishing effects due to water presence in localized inclusions would serve to better understand of fundamental properties of localized chromophore domains in diffusive media.

Here, we provide the first experimental evidence of a 2D angular photon distribution produced by a cylindrical void region with a clear identification of its spectroscopic signatures. The simple idealized system (homogenous turbid medium with a localized transparency) was modeled with void consisting of a water filled capillary immersed into an Intralipid-1% suspension. In-plane light detection was achieved by radiance measurements when a side firing fiber with a well-defined angular aperture was rotated around its axis working in a collection mode and probing the photon distribution. A combination of white light illumination with a broad-band spectroscopic detection allowed a spectral analysis. To detect the void region in its turbid background, we applied the spectro-angular approach previously used for homogenous medium characterization [10]. We demonstrate the utility of this approach here by expanding its application to a non-homogenous system.

2. Methods and materials

2.1. Setup for radiance measurements in Intralipid-1%

The details of the experimental setup have been presented elsewhere [9,10]. The conceptual top-view diagrams illustrating the measurement principles of two types of experiments performed in the work are shown in Figs. 1(a) and 1(b). The phantom Lucite box (with blackened 18-cm walls) was filled with Intralipid-1% suspension and accommodated a fiber with 800-micron spherical diffuser (Medlight, Switzerland) connected to a white light source and a 600-micron side firing fiber, the radiance detector (Molex/Polymicro Technologies, USA) with ~10° acceptance cone as indicated in Fig. 1. A side-firing fiber is produced by polishing a fiber tip at ~40.5° angle that provides a total internal reflection at the glass/air interface. When coupled to a light source, such fiber would emit light sideways (hence, the name) at the angle close to 90°. The fiber can also be used for (side) light collection as in the current work. The size of the box ensured an infinite medium geometry approximation. A 3.5-mm diameter thin-wall quartz capillary tube filled with water (~0.8 mL, ~6 cm immersion length) served as a non-scattering (void) target. For angular experiments, the source-detector separation was kept constant at 12 mm, and the target was positioned 6-mm away from the detector at one of the selected angles relative to the source-detector direction: 0°, 45°, 90°,
135° and 180° (as seen in Fig. 1(a)) with 0° angle corresponding to the direction to the light source. Due to the rotational symmetry of the setup, these angles would capture the essence of all angular related effects. For distance dependent experiments, the source-detector separation was kept constant at 34 mm, and the target was translated with 2 mm increments from 5 to 25 mm away from the detector keeping it in line between the source and detector (i.e., at 0° angle) as shown in Fig. 1(b). In all experiments, for every target position a set of radiance profiles was acquired by rotating the detecting fiber over a 360° range with a 2° step. The detecting fiber was connected to a USB 4000 spectrometer (Ocean Optics, USA) that collected a spectrum at every angular step. Intralipid-1% suspension was prepared by volume dilution of Intralipid-20% stock (Sigma Aldrich Canada).

Fig. 1. Conceptual top-view diagrams illustrating the principle of radiance measurements of a void region in a liquid phantom for a) angular experiments, b) distance dependent experiments.

2.2. Spectro-angular mapping approach

The spectro-angular mapping approach introduced earlier for a homogenous medium [10] can be further extended to analyze media with inclusions. Spectro-angular mapping doesn’t require a priori knowledge of spectral characteristics of a localized inhomogeneity nor its spatial location.

The approach relies on obtaining two data sets, one for the phantom without the inclusion and the other for the phantom with the inclusion. Individual spectra (i.e., radiance profiles) collected at various radiance detector angles in 0°-360° range in the phantom without inclusion are assembled into “the Intralipid matrix”, $I_{\text{Intralipid}}$ with columns corresponding to wavelengths and rows to angles. Placing the target at a certain distance from the detector along a selected angle and repeating the measurements yields “the Intralipid+target matrix”, $I_{\text{Intralipid+target}}$. An element by element ratio of two matrices, $I_{\text{Intralipid}}/I_{\text{Intralipid+target}}$ minimizes contributions from Intralipid, white light source and illuminating/detecting fibers, highlighting the target signal in a particular background. The value of the ratio indicates whether there is an increase (< 1) or a decrease (>1) in the directional photon density for the medium with the inclusion relative to the plain background. The ratio is referred as the Radiance Extinction Ratio (RER) in the current article and can be presented via surface or contour plots demonstrating intensity and spectral variations vs. angle. Such plots map the photon distribution in the angular domain, and hence are termed as spectro-angular maps. The information content of the plots provides important details about spectral and spatial properties of the void regions.
3. Results and discussion

3.1. Angular experiments: varying inclusion positions around detector

Water is a non-scattering and weakly absorbing medium, while Intralipid-1% is a highly scattering medium that has some absorption signatures of lipid particles and water. (Absorption of Intralipid-1% is much larger than water absorption for wavelengths < ~600nm but gradually approaches water absorption in 600-710 nm spectral range being equal above 710 nm [10].) When a water-filled capillary is inserted into the Intralipid medium, water replaces the equivalent volume of Intralipid and creates a localized non-scattering (void) region. (To test a possible effect of the thin wall capillary tube, the tube was filled with the same solution as the background, i.e. Intralipid-1% and placed at various distances and angles relative to the detector. No signal could be detected in such arrangements.)

Contour and surface plots of the RER for the water target located between the source and the detector (i.e., at 0° as in Fig. 1(a)) are shown in Figs. 2(a) and 2(b), correspondingly. The extinction ratio covers a range from ~0.8 to ~0.94 for all wavelengths and angles. It indicates that the radiance signal measured with the water inclusion present is higher than the one measured for the blank Intralipid-1%. Figure 2(a) shows the axial symmetry of the pattern relative to ~10° angle rather than 0°, indicating a likely positional misalignment of the target void. Note that the location of the target marked by a dashed line doesn’t coincide with the maximum of the radiance extinction ratio (~0.94, red zones) that is reached in the backscattering direction. It means that introducing the void region between the source and detector increases the photon density in all angular directions (i.e., all ratio values below 1) however the biggest increase happens in the direction of the inclusion (along 0°). Thus, the signature of the void region for this measurement geometry is manifested as a dip or valley along its location at 0°. Indeed, because of a preferential forward scattering of Intralipid-1% (anisotropy factor values 0.85-0.65 in the spectral range of interest [10]), introducing a non-scattering cavity will further increase the density of photons propagating in the forward direction.

Figure 2(b) emphasizes the spectral shape of the contour plot. To interpret the spectral behavior it is instructive to think in terms of a simple comparison of a fixed volume of water confined by the tube with an equivalent volume of the Intralipid. Both contain water, but the reference also contains lipid particles. Therefore, in the spectral range where water signatures are more prominent (starting from absorption peak at 740 nm and above), “more-water-and-less-lipid” medium would show an increasing deviation from the reference level (extinction...
ratios ~1), as seen above 700 nm in Fig. 2(b). For wavelengths <500 nm the medium with “more-water-and-less-lipid” will show an increasing deviation from unity due to a lack of absorption and Mie scattering from lipid particles present in the reference medium. Figure 2(a) suggests that one can achieve ~9% photon density increase (relative to the reference medium) confined to the direction of the sample in 500-700 nm range and ~20% increase in 850-900 nm range. This may open interesting applications of void engineering by positioning artificial void regions in strategic locations inside turbid media that would support higher dosages of light delivered to targeted areas. For example, in prostate applications, it would be interesting to explore the effect of small volume (<1 mL) or even larger volumes (e.g., ‘hydro-dissection’) interstitial saline injections on light distribution inside the organ.

![Fig. 3. Contour plots of RER of water target positioned at: a) 45°, b) 90°, c) 135°; d) 180° in Intralipid (marked by a dashed line) vs. radiance detector viewing angle and wavelength. All: source-detector separation 12 mm, detector-target separation 6 mm (experimental geometries as in Fig. 1(a) for 45°, 90°, 135° and 180° marked target locations).](image)

Spectro-angular maps for all other target positions, 45°, 90°, 135° and 180° in Intralipid-1% (when the target is not located between the source and detector) are shown in Figs. 3(a), 3(b), 3(c), and 3(d), respectively. As oppose to 0° target position, a common feature of these plots is that all values of the RER are >1 indicating that the void target reduces photon density in all directions relative to the reference. When behind the target (e.g., 0°), the detector senses increased directional density of photons propagating through the target. When looking at the target from other angles, the detector registers a decreased signal because locally reduced scattering will deliver less photons to the detector from the direction of the target. Let’s
consider the plot corresponding to a 90° target location first (Fig. 3(b)). The measurement geometry is shown in Fig. 1(a) with the target location marked "90°". The angular position of the sample marked by a dashed line coincides with the area of the maximum extinction. As the target is moved from 90° toward 180° position (Fig. 1(a)), the region of the maximum extinction follows the location of the target (Figs. 3(c) and 3(d)). Placing the target at 45° angle corresponds to some intermediate case between 0° and 90° target signatures (Fig. 3(a)). When moving the target from 0° to 90° position, the valley surrounded by two maxima as in Fig. 2(a) has to transform to a single maximum. Hence, 45° position corresponds to a case when two maxima are disappearing but the extinction along the target’s direction hasn’t assumed the maximal value yet. Such an intermediate target location presents a difficulty in identifying its exact angular position.

3.2. Distance dependent measurements: varying inclusion positions along the source-detector axis

For 12-mm source-detector separation used in the section 3.1, we couldn’t obtain enough data points when translating the 3.5-mm diameter target between the source and detector. However, positioning the target 4 mm away from the detector (the shortest distance for the setup) produced the clearest possible spectro-angular signature of the target (Fig. 4(a)).

![Image](image_url)

Fig. 4. a) Contour plot of RER of water target in Intralipid at 4-mm detector-target separation at 0°, 12-mm source-detector separation; b) polar plot of RER for the wavelength of 550 nm extracted from (a); c) contour plot of RER of water target in Intralipid at 5-mm detector-target separation at 0°, 34-mm source-detector separation; d) contour plot of RER of water target in Intralipid at 25-mm detector-target separation at 0°, 34-mm source-detector separation.
valley became more deeper accompanied by an overall increase in the dynamic range of the RER (0.72-0.94) indicating that target detectability depends on a proximity to a detector. A polar plot of RER for 550 nm (extracted from Fig. 4(a)) is shown in Fig. 4(b).

To obtain detailed distance dependent measurements the source-detector separation was enlarged to 34 mm and the target was translated with 2-mm increments from 5 to 25 mm away from the detector keeping the target fixed at 0° angle (Fig. 1(b)). The initial spectro-angular map corresponding to a 5-mm target position is shown in Fig. 4(c). The pattern remains similar to a 12-mm separation as in Fig. 2(a). It appears that target illuminating conditions do not play a critical role in its detectability. However, with the target moving away from the detector the valley starts to close and after ~9 mm it disappears completely. Instead, spectro-angular plots demonstrated quasi-continuous spectral bands as can be seen in Fig. 4(d) that displays the contour plot corresponding to a final 25-mm detector-target separation. The angular location of the void region can’t be determined from Fig. 4(d). Given the monotonous trend in the angular spread with distance, we expect it to continue all way toward the source even though we don’t have symmetric data close to the source. Thus, we expect that the angular resolution would be the best when the inclusion is closer to the detector and will deteriorate continuously when moving the inclusion toward the source. Positioning the target closer to the source and further from the detector will tend to spread detected photons more uniformly over the entire 360° range losing a sense of their origin as can be seen from Fig. 4(d).

By comparing the maximum and the minimum RER values for a particular wavelength, detectability of the inclusion can be quantified via the contrast value (e.g., (max - min)/max 100%). For the polar plot on Fig. 4(b), the contrast value is ~4% and the noise level is ~0.3%. Similar calculations performed for the wavelength of 550 nm from the plot from Fig. 4(c) produce the contrast value of ~1.3% with the noise level of ~0.3%. The contrast decreases and noise increases with moving the inclusion away from the detector and increasing the inclusion-source separation. Thus, for the inclusion located at a distance of 9 mm from the detector the contrast value drops down to the noise level (~0.6%), and the ability to localize the inclusion in the angular domain is lost. Any increase in scattering of the inclusion’s medium will also reduce contrast values limiting the detection of weakly-scattering inclusions.

The contour plot in Fig. 5(a) demonstrates the variations of the RER with changing the detector-target separation from 5 to 25 mm for all measured wavelengths along the actual direction of the void region (i.e., 10°) for 34 mm source-detector separation. An interesting
feature of the plot is that values of the RER for all wavelengths form a pattern—they start rising as the target moves away from the detector, reach the maximum close to a midpoint (17 mm) and then start decreasing pass that point. Due to a limit of a translation stage we couldn’t move the target beyond 20-mm distance which prohibited us from covering the entire 34-mm range. Thus, the first (5 mm) and the last (25 mm) target positions are not symmetric relative to the mid-point. (However, 9-mm and 25-mm target positions are symmetric relative to the mid-point.) The pattern suggests that an increased photon density is larger in the vicinity of the source and detector and smaller in the middle. In order to visualize the photon distribution in the angular domain, the RER was plotted vs. angle for the representative wavelength of 650 nm as shown in Fig. 5(b) (similar behavior was observed for all other wavelengths).

Several important observations can be made from Fig. 5(b). First, the plot shows a higher signal near the source and detector with a minimum between them. Second, the minimum occurs exactly in the middle between the source and detector. Third, the plot demonstrates that the ability to resolve the inclusion in the angular domain changes with distance, it is not symmetric relative to the midpoint and it continuously deteriorates with distance from the detector. A curvature of the contour lines in vicinity of the detector (a blue region) is a direct consequence of a presence of the minima for individual spectro-angular plots as seen in Fig. 4(c). Thus, the inclusion can be localized at 0° for 5 and 7 mm distance from the detector while starting from 9 mm the individual spectro-angular plots begin to look like the one shown in Fig. 4(d) which produces uniform color bands running parallel to the X-axis in Fig. 5(b). Hence, for detector-target separations above 9 mm an ability to localize the target in the angular domain is lost.

The origin of the increased photon density in the sample deserves a clarification. If the inclusion has the same absorption coefficient as the background but a lower scattering coefficient, then the total photon budget in the system remains unchanged (even though local photon densities will be modulated by the variations in local scattering properties). Lower local scattering will result merely in photon re-distribution such that a local increase and decrease in extinction ratio values will be observed in various directions in the system. If the inclusion has lower absorption in addition to lower scattering, it will result in the overall increase in the number of photons in the system leading to a positive photon budget. Local measurements in various locations in the system will still reveal a relative increase and decrease of the photon density. As was mentioned earlier, water inclusion corresponds to the second case because it doesn’t have absorption due to lipid particles in addition to significantly reduced scattering. Since we perform only local radiance measurements we can’t discriminate between similar manifestations for two cases without proper modeling.

3.3. Modeling the inclusion: a simple perturbation approach

Introducing a localized inclusion with optical properties different from the surrounding medium produces a perturbation that changes the photon density distribution. Such perturbation model for radiance has yet to be developed. However, as was shown in [11,12] by moving a point absorber or scatterer between the source and the detector on can obtain photon path distributions for fluence. Such perturbation approach then leads to the photon-path density distribution function that produces commonly known banana-shape patterns [11,12]. In a broader aspect, when other optical parameters like scattering coefficient, refractive index, scattering anisotropy, fluorescence etc., are used in the perturbation, a more formal and rigorous quantity can be introduced - the photon measurement density function (PMDF) [5,13]. Our radiance extinction ratio can be more easily interpreted with a simple perturbation approach. Following [11], the radiance measured in our system with the inclusion can be thought as radiance from the homogenous system plus radiance from the inclusion, i.e. $I_{\text{Intralipid+target}} = I_{\text{Intralipid}} + I_{\text{target}}$. Then, the RER can be rearranged as $I_{\text{Intralipid}}/I_{\text{Intralipid+target}} = I_{\text{target}}/(I_{\text{Intralipid}} + I_{\text{target}}) = 1/(1 + I_{\text{target}}/I_{\text{Intralipid}})$, where $I_{\text{target}}/I_{\text{Intralipid}}$ corresponds to a relative (normalized) perturbation caused by a target of a specific size. Thus, our experimental approach offers a measure of a relative radiance-based perturbation of a finite size target that differs by absorbing or scattering properties from the surrounding medium.
It is known that banana-shape patterns exhibit higher photon densities in proximity to the source and detector with a minimum mid-way between a source and a detector for fluence measurements [11,12]. Our results from Figs. 5(a) and 5(b) showing a larger increase in the RER closer to the source and detector with a smaller increase in the middle are therefore consistent with a known photon density distribution between the source and detector. The plot from Fig. 5(b) can be regarded as a relative angular photon density distribution function for radiance probed by a scattering perturbation at a selected wavelength.

To go beyond a simple conceptual picture explaining the spectral shape of the RER in section 3.1, we calculated ln(1/RER) for various detector-target separations for 0° (Fig. 6, lines, left axis). The lines follow a familiar pattern: highest values are achieved closer to the detector (5 mm) with a subsequent decrease with lowest values at the mid-point (17 mm) following by a rise toward the source (25 mm). The plot also shows the effective attenuation coefficient, μeff(λ) of Intralipid-1% (symbols, right axis [10]). The effective attenuation coefficient quantifies the combined effect of optical absorption and scattering to the total attenuation of light in turbid media. Both ordinate axes share the identical numerical scale. All lines demonstrate very similar shape and this shape is almost identical to the one of μeff(λ) for Intralipid-1%. When the tube with water is introduced inside Intralipid, it displaces an equivalent volume of Intralipid. Therefore, RER essentially compares optical properties of two samples: water filled capillary and the identical virtual capillary filled with Intralipid, both immersed into Intralipid. Reversing the ratio, taking a natural logarithm of it and assigning water to be the reference medium, rearranges it to the form used for absorption measurements, ln(I0/IIntralipid) where I0 corresponds to the radiance signal measured with water and IIntralipid to that with Intralipid. Measuring absorption in bulk multiple scattering medium requires a modified form of the Beer-Lambert law, \[ \ln(I_0 / I) = \mu_a(\lambda)L + G, \] where I0 is the intensity of incident light, I is the detected intensity after passing through the medium, \( \mu_a(\lambda) \) is the absorption coefficient of the medium, L is the total mean pathlength through the medium and G is a geometry factor that accounts for a contribution from scattering [1,14].

Measurements in our experimental setup can be considered analogous to those used in cuvette-based spectroscopy with few major modifications: 1) the sample has a cylindrical shape, 2) the sample is immersed into the turbid medium, so both illumination and detection occurs through it, 3) the sample is illuminated from all directions, 4) while the reference is the actual water filled capillary, the sample under study is the background material in a virtual capillary, 5) due to multiple scattering and absorption of the background, only a portion of photons incident on the sample is collected by a detecting fiber. Hence, the incident intensity equals to the radiance measured with the water column. The effective attenuation coefficient
from the diffusion theory accounts explicitly for both absorption and scattering as a combined loss mechanism in the target (opposite to a separate treatment in the modified Beer-Lambert law). Since the expression for radiance (as well as for fluence) contains a dependence on \( \mu_{\text{eff}}(\lambda) \) [10], it is not surprising to see a proportionality of the radiance extinction ratio to \( \mu_{\text{eff}}(\lambda) \) as in Fig. 6. The proportionality constant can be considered as a weighting factor accounting for a photon path distribution in turbid media leading to the following empirical relation:

\[
\ln\left(\frac{1}{\text{RER}}\right) = \ln\left(\frac{I_{\text{Intralipid-target}}}{I_{\text{Intralipid}}}\right) = \ln\left(\frac{I_0}{I_{\text{Intralipid}}}\right) = \mu_{\text{eff}}(\lambda) \cdot d \cdot K(\lambda)
\]

where \( d \) is the diameter of the tube (3.5 mm) and \( K \) is the weighting factor. The condition of \( K = 1 \) corresponds to a case when the detecting medium doesn’t affect the measured value. When detection occurs through a turbid medium some photons are lost due to absorption and some due to scattering producing an apparent increase in the effective attenuation coefficient. To take it into account and obtain the correct value of \( \mu_{\text{eff}}(\lambda) \), the weighting factor \( K(\lambda) \) can be introduced. Note, that the angular dependence is implicitly present both in left and right parts (e.g., in \( K \) of Eq. (1)) since the weighting factor can be mapped in the entire domain where measurements are performed. The particular example is given for 0° angle.

Following Eq. (1) and using experimental data for the RER and \( \mu_{\text{eff}}(\lambda) \) from Fig. (6), we calculated values of \( K \) for selected wavelengths at various detector-target separations (Fig. 7(a)). Values of \( K \) cover a range from about 0.01 to ~0.47 and tend to rise toward the detector and source with a minimum at the mid-point. (Note that the plot doesn’t show values of \( K \) returning to initial high values with distance because of a limited range of translation stage). However, due to noise presence in data it is difficult to discern the spectral behavior of \( K(\lambda) \). To make data more amenable to analysis, we presented it in a form of a spectro-spatial contour plot (Fig. 7(b)). It appears that closer to the detector there is almost no spectral dependence or at least it’s not well resolved in our data. As the target is translated toward the midpoint, the values of \( K \) for the spectral range 500-700 nm appear to show consistently lower values than outside of it indicating a possible spectral dependence.
to 0.06-0.07 in the middle. While a direct comparison with our values is not justified because of differences in the background material and inclusion’s properties, nevertheless the range of values and their spatial distribution is very similar to ours. With developments of the perturbation model for radiance, a direct verification of our empirically introduced weighting factor would become possible.

4. Conclusion

We present a radiance-based spectro-angular mapping approach for experimental detection and characterization of non-scattering inclusions (voids) in turbid media. We also introduced radiance extinction ratio (RER) that represents the ratio of the signal measured without the void to the signal measured with the void present, thus linking this metric to the void induced perturbation. Preferential forward scattering in Intralipid-1% increases density of photons measured behind the void (in the light source-void direction), while for all other directions a reduction in local photon density is observed producing specific signatures in measured spectro-angular maps. Positioning voids in strategic locations in turbid media may open a new way to light manipulation by creating increased photon densities in targeted areas. We showed that for void localization in the angular domain, proximity to the detector (not to the source) is important. It may help when designing measurement geometries. The spectroscopic signature of the void mimics the shape of the effective optical attenuation of Intralipid-1%. In order to increase utility of the radiance approach, we introduced an empirical proportionality constant between experimentally obtained radiance extinction ratio and the affective attenuation coefficient. It can serve as the first step in recovering optical properties of inclusions immersed in turbid media. In the formalism of the radiance extinction ratio approach, the signature of the void is thus expressed through a contribution of a virtual column of Intralipid displaced by the immersed water capillary.

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