Sequential estimation of optical properties of a two-layered epithelial tissue model from depth-resolved ultraviolet–visible diffuse reflectance spectra

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A method for estimating the optical properties of two-layered media (such as squamous epithelial tissue) over a range of wavelengths in the ultraviolet–visible spectrum is proposed and tested with Monte Carlo modeling. The method first used a fiber-optic probe with angled illumination and the collection fibers placed at a small separation (≈300 μm) to restrict the transport of detected light to the top layer. A Monte Carlo-based inverse model for a homogeneous medium was employed to estimate the top layer optical properties from the measured diffuse reflectance spectrum. Then a flat-tip probe with a large source-detector separation (≈1000 μm) was used to detect diffuse reflectance preferentially from the bottom layer. A second Monte Carlo-based inverse model for a two-layered medium was applied to estimate the bottom layer optical properties, as well as the top layer thickness, given that the top layer optical properties have been estimated. The results of Monte Carlo validation show that this method works well for an epithelial tissue model with a top layer thickness ranging from 200 to 500 μm. For most thicknesses within this range, the absorption coefficients were estimated to within 15% of the true values, the reduced scattering coefficients were estimated to within 20% and the top layer thicknesses were estimated to within 20%. The application of a variance reduction technique to the Monte Carlo modeling proved to be effective in improving the accuracy with which the optical properties are estimated. © 2006 Optical Society of America

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1. Introduction

A promising technique under development for squamous epithelial precancer and cancer detection is optical spectroscopy. Optical techniques offer several benefits over traditional diagnostic methods that include visual inspection (through a microscope or endoscope), followed by biopsy. Light can nondestructively interact with a large number of biological molecules intrinsically present in tissues, thus providing a wealth of biochemical and structural information related to disease progression, without the need for tissue removal. Additionally, advances in sensitive detectors and optical fibers make it possible to measure optical signals rapidly and remotely from human tissues in vivo.

There are a large number of absorbers in epithelial tissues in the ultraviolet–visible (UV–VIS) spectral range. The primary absorbers within the cells in the epithelium (top layer of the epithelial tissue) are tryptophan, reduced nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide (FAD).1 The primary absorber in the underlying stroma is hemoglobin.2 The primary elastic scatterers in the epithelium are cellular and subcellular components including nuclei and mitochondria, while the primary elastic scatterer in the stroma is collagen.3

It has been shown that the endogenous absorption and scattering contrast in precancers and early cancers of stratified squamous epithelial tissues, such as the cervix, varies with depth. Previous microscopy studies on cervical tissue slices4,5 and blocks6 show an increase in the contribution of NADH (source of absorption) within the epithelium, and a decrease in stromal collagen content (source of scattering) with...
the progression of neoplasia. Collier et al.\textsuperscript{7} have reported increased scattering in the epithelium of precancerous cervical tissues relative to that of normal tissues using confocal microscopy techniques. Moreover, there is increased blood vessel growth (source of absorption) in the stroma with cervical neoplasia, and this has been used by physicians during colposcopy to diagnose cervical precancers.\textsuperscript{8} Diffuse reflectance spectroscopy provides a measure of tissue absorption as well as scattering. Based on the findings described above, depth-dependent absorption and the scattering properties (optical properties) of tissues extracted from diffuse reflectance spectra could offer diagnostically useful information for the detection of epithelial precancers and early cancers.

Previously a number of light transport models\textsuperscript{9–15} were developed to compute optical properties from the diffuse reflectance spectrum measured from a homogeneous medium. However, since squamous epithelial tissues have a layered structure, the use of these simplistic models can cause significant errors in the extracted optical properties.\textsuperscript{16} Light transport models for two-layered media can potentially overcome the intrinsic weakness of homogeneous models of light transport. Several groups have extended the diffusion theory to calculate the optical properties of a two-layered medium.\textsuperscript{17–23} However, diffusion theory is not valid for highly absorbing media or for small source–detector separations, as is the case in the UV–VIS spectral range. Several other groups\textsuperscript{24,25} have proposed models based on Monte Carlo or hybrid methods. Hayakawa et al.\textsuperscript{24} developed a perturbation Monte Carlo method to estimate the optical properties of a two-layered medium, in which the perturbation in photon trajectories caused by a small amount of variation in the optical properties relative to baseline values was used to guide a nonlinear optimization algorithm for the estimation of optical properties. The perturbation approach is limited in that it is constrained to small changes in the optical properties (<30\% of baseline values for the scattering coefficient), and it requires that the baseline optical properties are known. Chang et al.\textsuperscript{25} proposed an analytical two-layered model to describe fluorescence spectra from epithelial tissues measured with a specific probe geometry, in which a single large fiber is used for both light excitation delivery and fluorescence emission collection. By assuming a low-scattering epithelium and a highly scattering stroma, as well as one-dimensional light propagation, Beer’s law was used to characterize light propagation in the epithelium while the diffusion theory was used to model light transport in the stroma. The analytical form of the model enables fast forward computation of fluorescence emission spectra. However, the presumed probe geometry and limited applicable range of tissue optical properties in each layer limits the utility of this model. Another disadvantage of previously published two-layer models in general is that they contain more free parameters compared to homogeneous models of light transport. The large number of unknowns can dramatically increase the computational time and/or even cause the inversion not to converge.\textsuperscript{19}

Fawzi et al.\textsuperscript{26} proposed an alternate sequential estimation approach for the determination of optical properties of a two-layered medium. In his approach, a flat-tip probe with a series of small source–detector separations was first used to measure spatially resolved reflectance from the top layer (thickness \(\geq 5\) mm), and a multivariate calibration model was used to extract the top layer optical properties. Then a flat-tip probe with a series of large source–detector separations was used to measure the phase delay and amplitude from both layers (using a frequency-domain technique), and the data were fitted to a two-layered frequency-domain diffusion model with the estimated top layer optical properties as known inputs. However, since this methodology is based on diffusion theory, it is not appropriate for use in the UV–VIS spectral range and/or for small source–detector separations.

We have adapted the general strategy of Fawzi et al.\textsuperscript{26} to extract the optical properties of a two-layered medium (such as squamous epithelial tissues) in the UV–VIS spectral range. Examples of squamous epithelial tissues are the cervix, skin, and oral cavity. Our approach combines an angled probe design and a flat-tip probe design to measure diffuse reflectance spectra preferentially from the top or bottom layer, respectively. The angled probe geometry has been shown effective in detecting fluorescence emission spectra\textsuperscript{27} and polarized reflectance\textsuperscript{28} selectively from superficial regions of tissue phantoms, while the flat-tip probe geometry is relatively more sensitive to deeper tissue volumes.\textsuperscript{29–31} A Monte Carlo-based light transport model for a homogeneous medium was used to extract the optical properties of the top layer from the diffuse reflectance spectrum obtained with the angled probe geometry. A second Monte Carlo model for a two-layered medium was used to extract the optical properties of the bottom layer and the thickness of the top layer from the diffuse reflectance spectra measured with the flat-tip fiber-optic probe given the known top layer optical properties. This sequential approach simplifies a large inverse problem with five unknowns (absorption and reduced scattering coefficients of both layers and the top layer thickness) into two small inverse problems, the first with two unknowns (absorption and reduced scattering coefficients of the top layer), and the second with three unknowns (absorption and reduced scattering coefficients of the bottom layer, and the top layer thickness). In this manner, the complexity of inversion is significantly reduced. The method was validated with independently simulated diffuse reflectance spectra.

2. Materials and Methods

Figure 1 shows a flow chart for sequential estimation of the optical properties of a two-layered medium, where parallelograms indicate input and output data, and rectangles represent processes. Arrows indicate the direction of data flow. Each component of the flow...
chart will be described in more detail. The probe designs and the simulations of diffuse reflectance will be discussed in Subsections 2.A and 2.B, respectively. The Monte Carlo models for homogeneous and two-layered media will be described in Subsections 2.C and 2.D, respectively.

A. Flap-Tip and Angled Probe Designs

Figure 2 shows the side views and the corresponding acceptance cones of the illumination and the collection fibers of (a) a flat-tip probe, (b) an angled probe design, and (c) a combination of the flat-tip and angled probe design. The arrows indicate light direction. In a flat-tip probe design [Fig. 2(a)], both illumination and the collection fibers are polished flat and placed perpendicular to a sample surface. In an angled probe design [Fig. 2(b)], either the illumination or collection fibers or both are polished obliquely and placed at an angle relative to the axis perpendicular to the sample surface.

Assuming an optically dilute sample, the geometric overlap between the illumination and the collection cones determines where photons travel before being detected. In Figs. 2(a) and 2(b), it is apparent that the depth of the overlap is much shallower for the angled probe [Fig. 2(b)] than that for the flat-tip probe [Fig. 2(a)]. This geometric rule also applies to a turbid medium when light paths are short, as is the case when angled probe geometry with a small source–detector separation is used. It should be noted that it has already been shown that angled probe geometries are sensitive to superficial tissue volumes, while flat-tip probe geometries are sensitive to relatively deeper tissue volumes. The complementary attributes of the two probe geometries make them ideally suited to selectively detect diffuse reflectance from the two distinct sublayers of epithelial tissues [Fig. 2(c)]. The angled probe can be used to detect diffuse reflectance from the superficial epithelial layer, while the flat-tip probe geometry can be used to detect diffuse reflectance primarily from the bottom stromal layer.

B. Simulation of Diffuse Reflectance Spectra for the Flap-Tip and Angled Probe Designs

A three-dimensional, weighted-photon Monte Carlo code written with the standard ANSI C programming language was used to simulate diffuse reflectance spectra obtained from a two-layered model of epithelial tissues using the flat-tip and angled probe geometries. The Henyey–Greenstein phase function was used in the Monte Carlo code in all the simulations unless specified otherwise. The diameter of all fibers was 100 \( \mu \)m and the numerical aperture (NA) was 0.22. The center-to-center distances between the il-
lumination and the collection fibers (source–detector separation) were varied from 100 to 2000 μm for both the angled and the flat-tip probe geometries. The tilt angle of the illumination and collection fibers relative to the axis perpendicular to the sample surface was either 0° for the flat-tip probe or 45° for the angled probe.

The top layer thickness of the tissue model was varied from 25 to 750 μm to cover a wide range of epithelial thicknesses, and the bottom layer thickness was set at 29 700 μm to represent a semi-infinite stromal layer. Figure 3 shows the absorption and reduced scattering coefficients of (a) the top layer and (b) the bottom layer of the two-layered epithelial tissue model as a function of wavelength that were used as inputs in the Monte Carlo simulations. These absorption and scattering coefficients were determined using Beer’s law and Mie theory, respectively, assuming known absorbers and scatterers (this is a requirement of the forward Monte Carlo models in Subsections 2.C and 2.D, which assume that there are known absorbers and scatterers in the tissue). The absorption coefficient of the top layer of the tissue model was assumed to be that of the absorber, Nigrosin (Sigma Chemical Company, St. Louis, Missouri) at a concentration of 0.006 mg/ml, and the scattering coefficient of the top layer was assumed to be that of the scatterer, polystyrene spheres (Polysciences Incorporated, Warrington, Pennsylvania) with a diameter of 1.053 μm at a volume concentration of 0.256%. The bottom layer was assumed to contain the same absorber and scatterer, but at concentrations of 0.018 mg/ml and 0.716% by volume fraction, respectively. Nigrosin was selected as the absorber because it is widely used in tissue phantom optical studies. Polystyrene spheres were selected as the scatterer because of their well-characterized scattering properties for use in the Mie theory. The components and concentrations were chosen such that the ranges of optical properties of the two-layered tissue model are similar to those of human cervical tissue. Although the shape of the absorption spectrum of Nigrosin is different from that of real cervical tissue, the methodology presented in this paper is equally applicable as long as the components in the turbid media are known, and the assumption is true that the top layer is semi-infinite for the angled probe geometry.

Ten million photons were launched in each simulation at random, uniformly distributed locations over an area defined by the illumination fiber size. The angular profile of incident photons followed a Gaussian distribution with the cutoff angle defined by the NA.38 The diffuse reflectance escaping the medium was collected over an area defined by the collection fiber size within a range of angles defined by the NA of the collection fiber. The refractive index of the medium above the tissue model was set to 1.45 to simulate an optical fiber and that of the tissue model was set to 1.37. A cylindrical coordinate system, in which the axial dimension is perpendicular to the top surface of the medium and the radial dimension corresponds to any direction perpendicular to the axial dimension, was applied to record quantities of interest for the flat-tip probe. The axial and radial grid sizes were 20 and 50 μm, respectively. A Cartesian coordinate system was used to record quantities of interest for the angled probe. The Cartesian coordinate system was set up in such a way that the side views shown in Fig. 2 are in the x–z plane, where the z axis corresponds to the axial dimension. The grid sizes in the x, y, and z dimensions were 50, 100, and 20 μm, respectively.

In order to visualize light distribution in the tissue model for detected photons, weighted fluence and weighted visiting frequency distributions were created. The term “fluence distribution” refers to the radiant energy propagating in all directions through a given location and is calculated by dividing the deposited photon weight within a local voxel by the local absorption coefficient. The contribution of the photon to the fluence distribution becomes smaller and smaller as it takes more and
more steps during its random walk in a turbid medium. Because of the attenuation upon each collision, the fluence distribution will tend to have a higher intensity closer to the light source, which does not weigh the contribution of each step of the photon random walk equally. To remove the distortion in the light distribution that is due to attenuation of the photon weight with each step, the concept of visiting frequency was introduced. The term “visiting frequency” refers to the number of times that photons visit a voxel divided by the total attenuation coefficient at the voxel. The visiting frequency distribution is essentially equivalent to the fluence distribution in the case of zero absorption, and thus is not influenced by the attenuation of the photon weight with each step that it takes.

The fluence and weighted visiting frequency distributions were multiplied by the survival weight of the photon to account for the fact that an exit photon with a larger survival weight contributes more to the total detected reflectance. The weighted fluence and weighted visiting frequency distributions were then divided by the number of incident photons and the volume of a single voxel to facilitate comparison across simulations. A more detailed explanation of the relationship between absorption, fluence, and visiting frequency distributions is provided in Appendix A.

The three-dimensional weighted fluence and visiting frequency distributions contain huge amounts of data and thus are difficult to visualize and interpret. To simplify the presentation of the data, the three-dimensional distribution was reduced to two dimensions by integrating data over the azimuthal angle in the cylindrical coordinate system for the flat-tip probe. Because of the radial symmetry of the ring collection area around the central axis of the illumination fiber in the flat-tip probe design, the two-dimensional distribution is equivalent to the three-dimensional distribution. However, a similar approach cannot be used for the angled probe design since the collection area is not radially symmetric around the illumination fiber. Instead, data reduction from three to two dimensions was achieved by including the data recorded only at a vertical longitudinal plane that passes through the central axes of both illumination and collection fibers. Figure 2(b) shows the resulting view when such a longitudinal plane is chosen for an angled probe. Because the angled probe geometry is symmetric about the longitudinal plane, the two-dimensional view obtained in this manner would be able to faithfully represent the three-dimensional data in terms of the sensing depth.

C. Monte Carlo-Based Model for Extraction of Optical Properties of the Top Layer in a Two-Layered Medium

A previously developed Monte Carlo model for a homogeneous medium was used to extract absorption and scattering coefficients from the diffuse reflectance spectra obtained from the top layer of the two-layered tissue model with the angled probe geometry.

The three-dimensional weighted fluence and visiting frequency distributions contain huge amounts of data and thus are difficult to visualize and interpret. To simplify the presentation of the data, the three-dimensional distribution was reduced to two dimensions by integrating data over the azimuthal angle in the cylindrical coordinate system for the flat-tip probe. Because of the radial symmetry of the ring collection area around the central axis of the illumination fiber in the flat-tip probe design, the two-dimensional distribution is equivalent to the three-dimensional distribution. However, a similar approach cannot be used for the angled probe design since the collection area is not radially symmetric around the illumination fiber. Instead, data reduction from three to two dimensions was achieved by including the data recorded only at a vertical longitudinal plane that passes through the central axes of both illumination and collection fibers. Figure 2(b) shows the resulting view when such a longitudinal plane is chosen for an angled probe. Because the angled probe geometry is symmetric about the longitudinal plane, the two-dimensional view obtained in this manner would be able to faithfully represent the three-dimensional data in terms of the sensing depth.

Figure 4 shows a flow chart of (a) the forward model and (b) the inverse model for estimation of the top layer optical properties. The figures were adapted from Ref. 34.
predicted reflectance. Then the sum of the square of the differences between the predicted and the actual measured reflectance spectra (i.e., sum of the square of errors) is computed. The input parameters are iteratively updated until the sum of the square of errors reaches a global minimum. A Gauss–Newton nonlinear least-squares algorithm provided in the MATLAB optimization toolbox (MathWorks, Incorporated, Natick, Massachusetts) was used for the minimization scheme.

In order to increase the efficiency of the Monte Carlo simulations (in the forward part of the model), a scaling approach previously described by Graaff et al.\(^4\) was incorporated such that only a single Monte Carlo simulation with an impulse incident beam needs to be run, the output of which can be scaled for a wide range of absorption and scattering coefficients. The parameters of the single Monte Carlo simulation were as follows: \( \mu_a = 0 \text{ cm}^{-1} \), \( \mu_s = 150 \text{ cm}^{-1} \), \( g = 0.9 \). The number of incident photons was \( 4 \times 10^7 \). In order to adapt this method for use with a probe geometry, convolution was used to integrate over the illumination and collection areas of the geometry to determine the probability that a photon, launched from a finite illumination area, would be collected within a specific collection area assuming spatial invariance.

D. Monte Carlo–Based Model for Extraction of Optical Properties of a Two-Layered Medium

A two-layered Monte Carlo model was then used to extract the optical properties of the bottom layer and the thickness of the top layer of the two-layered tissue model, from the diffuse reflectance spectrum obtained with the flat-tip probe geometry. This model assumes that the optical properties of the top layer are known from the previous step. In the forward model, Beer’s law was used to calculate the absorption coefficient of the bottom layer given the absorber concentration and wavelength-dependent extinction coefficient; Mie theory was used to determine the scattering coefficient of the bottom layer given the scatterer size, density, and refractive index mismatch. Next the optical properties of the two layers and the thickness of the top layer were used as inputs into the two-layered Monte Carlo model to generate a predicted diffuse reflectance. The inversion approach used was the same as that described in Subsection 2.C, except that the optical properties being retrieved were for the bottom rather than the top layer, and the thickness of the top layer was included as an additional free parameter.

A different approach was used to increase the efficiency of the two-layered forward Monte Carlo model. Specifically, a series of baseline Monte Carlo simulations were run ahead of time to generate a database of diffuse reflectance values for a two-layered medium for zero absorption and a wide range of scattering coefficients\(^3\) of the top and bottom layers. For each scattering coefficient pair (top and bottom layers), simulations were carried out for a range of thicknesses. Table 1 lists the reduced scattering coefficients and thicknesses of the top and bottom layers used in the baseline simulations to generate the Monte Carlo database for two-layered media. The anisotropy factor was 0.9, and the absorption coefficient was zero in all simulations. All combinations of these parameters were simulated.

| Table 1. Scattering Coefficients and Thicknesses of the Top and Bottom Layers Used in the Baseline Simulations to Generate the Monte Carlo Database for Two-Layered Media\(^a\) |
|-----------------|-----------------|-----------------|
| Reduced scattering coefficient, \( \mu'_s \) (cm\(^{-1}\)) | Top Layer | Bottom Layer |
| 3, 3.67, 4.48, 5.48, 14, 17, 11, 20, 91 | 6.69, 8.18, 25,56, 31,24, 10,00, 12,22, 38,18, 46,67 |
| 14,94 | 14,94 |
| Thickness (\( \mu m \)) | 0, 50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800 | 30,000 |

\(^a\) The anisotropy factor was 0.9, and the absorption coefficient was zero in all simulations. All combinations of these parameters were simulated.

3. Results

Figure 5 shows longitudinal views of (a) the weighted fluence distribution and (b) the weighted visiting frequency distribution at 420 nm for the angled probe (source–detector separation of 300 \( \mu m \)), as well as (c) the weighted fluence distribution, and (d) the weighted visiting frequency distribution at 580 nm for the flat-tip probe (source–detector separation of 1500 \( \mu m \)) within the two-layered tissue model. The top layer thickness in the two-layered model is 300 \( \mu m \). The rationale for selecting the wavelengths of 420 and 580 nm is that they correspond to the smallest and largest absorption coefficients, respectively, over the wavelength range of interest (see Fig. 3). At 420 nm, the light is expected to penetrate deepest (worst case for the angled probe), while at 580 nm light penetration is expected to be shallowest (worst case for the flat-tip probe).

Figures 5(a) and 5(b) indicate that the light distri-
bution is confined to the top layer for the angled probe in both distributions, suggesting that the assumption of a semi-infinite homogeneous medium is appropriate when extracting optical properties of the top layer from diffuse reflectance obtained with the angled probe. In Figs. 5(c) and 5(d), the light distribution overlaps with the bottom layer for the flat-tip probe in both types of distribution, although this is particularly evident in the weighted visiting frequency distribution. The weighted fluence and visiting frequency distributions of the tissue model obtained with the angled probe at 660 nm (smallest scattering coefficient) and with the flat-tip probe at 360 nm (largest scattering coefficient) showed similar trends (figures not shown here).

Table 2 shows the sensitivity of the angled probe to the top layer (for a top layer thickness of 300 μm) based on the fluence and visiting frequency distributions at two wavelengths, 420 (smallest absorption coefficient) and 600 nm (smallest scattering coefficient). These quantitative metrics confirm the validity of our design assumptions, i.e., the angled probe is mainly sensitive to the diffuse reflectance from the top layer.

Figure 6 shows the distribution of the exiting photons’ survival weights for the two-layered tissue model (for top layer thickness of 300 μm) at (a) 420 nm (smallest absorption coefficient), simulated for the angled probe, and (b) 580 nm (largest absorption coefficient), simulated with the flat-tip probe. Also shown are the exiting photons’ survival weights from corresponding homogeneous tissue models with the optical properties of the top (for the angled probe) or bottom (for the flat-tip probe) layers for purposes of comparison. A total of 50 equally spaced weight bins
that cover [0, 1] was used to count the number of photons whose weights fall within these bins. The counts were then divided by the total number of incident photons to obtain a probability distribution.

Figure 6(a) shows that the photons with large survival weights are collected by the angled probe, while Fig. 6(b) shows that photons with much smaller survival weights are collected by the flat-tip probe. The photon survival weight distributions simulated for the two-layered tissue model and the corresponding homogeneous tissue model (with the optical properties of the top layer) using the angled probe are almost identical, which confirms that the angled probe mainly examines the top layer. The photon survival weight distributions simulated for the two-layered tissue model and the corresponding homogeneous tissue model (with the optical properties of the bottom layer) using the flat-tip probe are similar; which suggests that the flat-tip probe preferentially examines the bottom layer.

Figure 7 shows the simulated diffuse reflectance spectra of the two-layered tissue model (thickness of the top layer is 300 μm) for (a) the flat-tip probe with a separation of 300 μm, (b) the flat-tip probe with a separation of 1500 μm, (c) the angled probe with a separation of 300 μm, and (d) the angled probe with a separation of 1500 μm. For purposes of comparison, the spectra simulated for corresponding homogeneous media with optical properties equal to those of either the top or bottom layers are also shown, which are, respectively, marked as “Homogeneous Top” and “Homogeneous Bottom” in the legends. A total of 50 equally spaced weight bins that cover [0, 1] was used to count the number of photons whose weights fall within these bins. The counts were then divided by the total number of incident photons to obtain a probability distribution.

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**Table 2. Sensitivity of the Angled Probe to the Top Layer**

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Optical Properties (cm⁻¹)</th>
<th>Sensitivity to the Top Layer Based on Fluence</th>
<th>Sensitivity to the Top Layer Based on Visiting Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>420</td>
<td>μ₂ₐ_top = 1.57</td>
<td>97%</td>
<td>89%</td>
</tr>
<tr>
<td></td>
<td>μ₂ₐ_top = 9.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>μ₂ₐ_bottom = 4.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>μ₂ₐ_bottom = 26.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>660</td>
<td>μ₂ₐ_top = 2.24</td>
<td>98%</td>
<td>92%</td>
</tr>
<tr>
<td></td>
<td>μ₂ₐ_top = 7.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>μ₂ₐ_bottom = 6.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>μ₂ₐ_bottom = 21.81</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The probe’s sensitivity to the top layer (for a top layer thickness of 300 μm) is based on the fluence or visiting frequency distributions at two wavelengths: 420 (smallest absorption coefficient) and 600 nm (smallest scattering coefficient).
between the two diffuse reflectance spectra in Fig. 7(b) implies that it is not possible to extract the optical properties of the bottom layer directly from the diffuse reflectance spectrum of the two-layered tissue model, measured by a flat-tip probe with a large source–detector separation as if the tissue were homogeneous, because the contribution from the top layer to the detected diffuse reflectance appears to be nonnegligible.

Figure 8 shows the actual and estimated absorption ($\mu_a$) and reduced scattering coefficients ($\mu_s'$) of (a) the top layer, and (b) and (c) the bottom layer in the two-layered tissue model (the thickness of the top layer is 300 $\mu$m). For the results shown in Fig. 8(b), the exact optical properties of the top layer were assumed as known in the inversion, while for Fig. 8(c) the estimated top layer optical properties shown in Fig. 8(a) were used in the inversion. Extraction of the top layer optical properties were based on the diffuse reflectance spectrum simulated for the angled probe with a source–detector separation of 300 $\mu$m, while extraction of the bottom layer optical properties were based on the diffuse reflectance spectrum simulated for the flat-tip probe with a source–detector separation of 1500 $\mu$m. In Fig. 8(a), the extracted absorption coefficients of the top layer are in excellent agreement with their actual values and the extracted reduced scattering coefficients are within 10% of their actual values. Comparison of Figs. 8(b) and 8(c) indicated that the errors in the estimated top layer optical properties did not significantly affect the accuracy of the estimated bottom layer optical properties.

Table 3 summarizes percent deviations of estimated optical properties for (a) the top layer and (b) and (c) the bottom layer. In case (b) the exact optical properties of the top layer were assumed as known in the inversion, and in case (c) the estimated top layer optical properties shown in Fig. 8(a) were used in the inversion. The percent deviations of the estimated reduced scattering coefficients were averaged over the wavelengths of interest, and the standard deviation was calculated to describe the variation in the percent deviations. The thickness of the top layer was varied from 200 to 500 $\mu$m. Most of the deviations in the estimated optical properties are smaller than 20%. It appears that the accuracies of the estimated optical properties are not considerably better when the top layer thickness is present in the Monte Carlo database (bold fonts in Table 3).

Figure 9 shows the percent deviation in the estimated top layer thicknesses as a function of the top layer thickness for the case in which the exact optical properties of the top layer were assumed as known in the inversion (open circles); and the case in which the estimated top layer optical properties were used in the inversion (star symbols). The horizontal line indicates zero deviation. It can be seen that the accuracy of the estimated top layer thicknesses is smaller than 20% for most thicknesses within a range from 200 to 500 $\mu$m. However, the data point with an actual thickness of 325 $\mu$m is an outlier. Again the accuracy of the estimated top layer thickness appears not to be considerably affected by the errors in the estimated top layer optical properties.

To test the sensitivity of the estimation accuracy to
4. Discussion

This study demonstrates a method for sequential estimation of the optical properties of a two-layered medium. The Monte Carlo validation shows that the estimated optical properties are within 20% of the true values for the two-layered medium with top layer thicknesses ranging from 200 to 500 μm. When the actual top layer thickness is out of this range, the estimation is considerably worse (results not shown). Specifically, the estimated top layer optical properties deviate significantly from their actual values when the top layer thickness is smaller than 200 μm; the estimated bottom layer optical properties, as well as the estimated top layer thickness, contain large errors when the top layer thickness is greater than 500 μm. The inaccuracy for small top layer thicknesses lies in the assumption of a semi-infinite top layer being invalid, which can be appreciated from Figs. 5(a) and 5(b). The inaccuracy for large thicknesses is perhaps because it is less likely for light to reach the bottom layer. A larger thickness of the top layer requires more incident photons to enable adequate sampling of the bottom layer. Therefore a Monte Carlo code that provides reduced variance between independently simulated results could help to address this issue.

The stochastic nature of Monte Carlo modeling requires a large number of incident photons to accurately estimate the values of random variables of interest. For a regular Monte Carlo simulation, the decrease in the standard deviation of a scored random variable is proportional to the square root of the number of incident photons. To remove this limit, a variance reduction technique that has been used extensively in the Monte Carlo modeling of neutron transport, called geometry splitting, was applied to the baseline Monte Carlo simulations as well as to independent test simulations to improve the match between reference and test data. The details of the geometry splitting technique and its implementation are discussed in Appendix B. The estimation of top and bottom layer optical properties was carried out in the same way as described previously for the two-layered tissue model with a top layer thickness of 325 μm, which is the worst case in Table 3. The results are shown in Fig. 10. It can be seen that the accuracy with which the top layer properties are esti-
The horizontal line indicates zero deviation.

Layer optical properties were used in the inversion (star symbols). In case (b) the exact optical properties of the top layer were known in the inversion, and in case (c) the estimated top layer optical properties shown in Fig. 8(a) were used in the inversion. The percent deviations of the estimated reduced scattering coefficients were averaged over the wavelengths of interest and expressed as mean ± std (standard deviation). The thickness of the top layer was varied from 200 to 500 μm. Bold fonts indicate those thicknesses present in the Monte Carlo database.

<table>
<thead>
<tr>
<th>(a) Top Layer Thickness (μm)</th>
<th>200</th>
<th>300</th>
<th>325</th>
<th>400</th>
<th>450</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deviation in μa (%)</td>
<td>14.8</td>
<td>-1.3</td>
<td>31.4</td>
<td>-12.6</td>
<td>-14.7</td>
<td>-3.7</td>
</tr>
<tr>
<td>Deviation in μa' (%)</td>
<td>-5.3 ± 0.3</td>
<td>-10.8 ± 0.4</td>
<td>-7.5 ± 1.2</td>
<td>-12.4 ± 0.3</td>
<td>-12.6 ± 0.3</td>
<td>-11.6 ± 0.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(b) Top Layer Thickness (μm)</th>
<th>200</th>
<th>300</th>
<th>325</th>
<th>400</th>
<th>450</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deviation in μa (%)</td>
<td>6.6</td>
<td>-2.2</td>
<td>10.4</td>
<td>-8.0</td>
<td>-4.5</td>
<td>-9.1</td>
</tr>
<tr>
<td>Deviation in μa' (%)</td>
<td>-5.2 ± 4.0</td>
<td>-5.0 ± 0.3</td>
<td>-4.9 ± 3.9</td>
<td>-11.2 ± 0.8</td>
<td>-5.2 ± 1.0</td>
<td>-25.7 ± 1.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(c) Top Layer Thickness (μm)</th>
<th>200</th>
<th>300</th>
<th>325</th>
<th>400</th>
<th>450</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deviation in μa (%)</td>
<td>4.6</td>
<td>-4.3</td>
<td>6.6</td>
<td>-8.7</td>
<td>-12.6</td>
<td>-6.5</td>
</tr>
<tr>
<td>Deviation in μa' (%)</td>
<td>-2.8 ± 4.1</td>
<td>-3.4 ± 1.5</td>
<td>-18.3 ± 3.0</td>
<td>-1.8 ± 0.8</td>
<td>-4.3 ± 1.5</td>
<td>-12.7 ± 1.0</td>
</tr>
</tbody>
</table>

The percent deviations of estimated optical properties were calculated for (a) the top layer and (b) and (c) the bottom layer. In case (b) the estimated was dramatically improved for both layers. The absorption coefficients were estimated to within 10% of the true values while the reduced scattering coefficients were estimated to within 5%.

The diffuse reflectance is expected to be sensitive to the phase function used for short source–detector separations. However, the main purpose of this paper is to demonstrate the methodology of the sequential estimation approach in the UV–VIS spectrum. This methodology should work with different phase functions as long as the assumption of the method—that the angled probe is mainly sensitive to the diffuse reflectance from the top layer—is valid. The authors have developed a Monte Carlo code that can simulate arbitrary phase functions, including the Mie phase function. According to the Monte Carlo results simulated with the Mie phase function, the sensitivity to the top layer reflectance for the angled probe is 87% at 420 nm and 91% at 660 nm for the theoretical epithelial tissue model employed in this study. This is similar to the sensitivity

![Fig. 9](image)

**Fig. 9.** Percent deviation in the estimated top layer thicknesses as a function of the layer thickness for the case in which the exact optical properties of the the layer were assumed as known in the inversion (open circles); and for the case in which the estimated top layer optical properties were used in the inversion (star symbols). The horizontal line indicates zero deviation.

![Fig. 10](image)

**Fig. 10.** Percent deviations of (a) estimated absorption coefficients and (b) estimated reduced scattering coefficients from their actual values. The legend “Regular MC” means that both the baseline simulations for the Monte Carlo database and the simulations of the two-layered tissue model were run with a Monte Carlo code without the geometry splitting variance reduction technique incorporated; the legend “Improved MC” means that these simulations were run with a Monte Carlo code with the geometry splitting technique built in. The category on the x-axis, “Bottom layer with known top layer properties,” means that the bottom layer optical properties were estimated with the exact top layer optical properties as known; the category, “Bottom layer with estimated top layer properties,” means that the bottom layer optical properties were estimated in the case where the top layer optical properties estimated in the previous step were used in inversion.
values (89% at 420 nm and 92% at 660 nm) obtained with the Henyey–Greenstein phase function at the same wavelengths. Thus the Henyey–Greenstein function seems to be a reasonable phase function to use in this study. In future studies the effect of phase function on the estimation accuracy will be studied more systematically.

It took approximately 30 s on average to carry out one nonlinear least-squares regression for the top layer, and less than 3 min for the bottom layer when running the inversion in MATLAB 7 on a personal computer with a Pentium-4 CPU at 2.4 GHz. Generally, 20–50 regressions with randomly chosen initial guesses were needed to ensure a global solution for the results shown in Figs. 8 and 9. The reduction of the variance in the Monte Carlo simulated results would help reduce the number of required regressions.

The angled probe design can be implemented in various ways. The simplest implementation would be the one shown in Fig. 2(b), in which both illumination and collection fibers are polished obliquely and placed at an angle relative to the normal axis of the sample surface. One advantage of this method is that the incident light profile and light collection parameters are straightforward to model in this case, which would greatly facilitate baseline Monte Carlo simulations. A similar implementation of the angled probe has been employed to selectively detect fluorescence from the superficial layer of a two-layered tissue phantom. Another implementation is to add a focusing lens between illumination and collection fibers that are polished flat and placed perpendicular to the sample surface. The light cones of illumination and collection fibers would converge after passing through the lens, which is equivalent to the function of an angled probe. A ball lens has been used for this purpose to detect fluorescence reflectance from superficial volumes of a two-layered tissue phantom.

5. Conclusions
A method for sequential estimation of optical properties of two-layered media over a range of wavelengths in the UV–VIS spectral range is proposed and tested with independent Monte Carlo simulations. The method first used a fiber-optic probe with angled illumination and collection fibers placed at a small separation (≤300 μm) to restrict light transport to the top layer. The fluence and visiting frequency distribution were used to validate the assumption of the top layer being viewed as semi-infinite. A Monte Carlo-based inverse model for a homogeneous medium was employed to estimate the top layer optical properties from measured diffuse reflectance spectra. Then a flat-tip probe with a large source–detector separation (≥1000 μm) was used to detect diffuse reflectance preferentially from the bottom layer. A second Monte Carlo-based inverse model for a two-layered medium was applied to estimate the bottom layer optical properties as well as the top layer thickness given that the top layer optical properties have been estimated. The number of unknown variables and the complexity of the inversion process were significantly reduced by this strategy. The results of Monte Carlo validation show that this method works well for an epithelial tissue model with a top layer thickness ranging from 200 to 500 μm. Surprisingly it appears that the errors in the estimated top layer optical properties do not significantly affect the accuracy of subsequent estimations, which is probably due to the relatively large variance between simulated results in the baseline database and those in the test data set. The geometry splitting technique was applied to reduce the variance in the Monte Carlo simulated data, which was found to be effective.

Appendix A
Figure 11 shows a schematic of the trajectory of a photon in a turbid medium. Let us assume that the absorption and scattering coefficients of the medium are μ_a and μ_s, respectively, and the initial weight of the photon is 1. Then the albedo is α = μ_s/(μ_a + μ_s). The mean free path of the random walk is related to 1/(μ_a + μ_s).

Table 4 lists the contribution of a photon to the absorption, fluence, and visiting frequency distribution at each collision in Fig. 11.

![Figure 11](image.png)

This figure shows a schematic of the trajectory of a photon in a turbid medium. The number 1, ..., N indicates the index of the collision. The area beneath the horizontal line represents the turbid medium.

<table>
<thead>
<tr>
<th>Index of Collision</th>
<th>Photon Weight Before Collision</th>
<th>Absorption</th>
<th>Fluence</th>
<th>Visiting Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1 – α</td>
<td>(1 – α)/μ_a = 1/(μ_a + μ_s)</td>
<td>1/(μ_a + μ_s)</td>
</tr>
<tr>
<td>2</td>
<td>α</td>
<td>(1 – α) α</td>
<td>[(1 – α) α/μ_s = (1 – α)/μ_s + μ_s]</td>
<td>1/(μ_a + μ_s)</td>
</tr>
<tr>
<td>i</td>
<td>α^{i-1}</td>
<td>(1 – α) α^{i-1}</td>
<td>[(1 – α) α^{i-1}/μ_s = α^{i-1}/(μ_s + μ_a + μ_s)]</td>
<td>1/(μ_a + μ_s)</td>
</tr>
<tr>
<td>N</td>
<td>α^{N-1}</td>
<td>(1 – α) α^{N-1}</td>
<td>[(1 – α) α^{N-1}/μ_s = α^{N-1}/(μ_s + μ_a + μ_s)]</td>
<td>1/(μ_a + μ_s)</td>
</tr>
</tbody>
</table>
dividing the photon weight deposited within a local voxel by the local absorption coefficient.9 Visiting frequency refers to the number of times a photon visits a voxel divided by the total attenuation coefficient of the voxel. The visiting frequency distribution for a particular set of optical properties, say \( \mu_a = \mu_{a0}, \mu_s = \mu_{s0} \), is equivalent to the fluence distribution for the corresponding set of optical properties, i.e. \( \mu_a = 0, \mu_s = \mu_{a0} + \mu_{s0} \). In other words, the visiting frequency is equivalent to the fluence distribution for zero absorption.

If \( \alpha < 1 \), the photon weight will attenuate rapidly after a number of collisions because of the power term shown in Table 4. As a result, the contribution of the photon to the absorption and fluence distribution becomes smaller and smaller as it takes more and more steps. These two distributions would suggest that the photon spent more time at the first and second collision sites than at the \( N \)th collision site. Because of attenuation upon each collision, the absorption and fluence distributions will tend to have a higher intensity close to the light source, which does not weigh the contribution of each step of the random walk equally.

To remove the distortion in the light distribution owing to the attenuation of the photon with each step, the concept of visiting frequency was introduced.

A smaller albedo \( [\mu_s/(\mu_a + \mu_s)] \) or a larger average number of collisions would cause greater differences between the fluence distribution and the visiting frequency distribution according to Table 4 because the effect of absorption would be more dramatic in these cases. The differences between the fluence distribution and the visiting frequency distribution for the same optical properties in the cases of different illumination-collection geometries can be seen in Fig. 5 in Section 3. In Figs. 5(a) and 5(b) there are no visible differences between the two distributions, because only a small number of collisions occurred for photons detected by the angled probe with a small source–detector separation (300 \( \mu \)m). In contrast, the flat-tip probe with a large separation (1500 \( \mu \)m) would collect photons that experienced many more collisions; therefore the difference between the two distributions in this case is more obvious. The weighted fluence distribution is apparently more concentrated toward the light source, while the weighted visiting frequency distribution is more uniform from the source region to the detector region. This makes more sense because the two regions should contribute equally to detected reflectance intuitively.

Appendix B

Geometry splitting is one of the oldest and most widely used variance reduction techniques in Monte Carlo modeling.43 In this technique, the target space is separated into several volumes. This technique attempts to increase sampling in important volumes and decrease sampling in unimportant volumes, thus saving time to achieve desirable variances in important volumes. Ideally the importance of a particular volume is proportional to the contribution of photons traveling in this volume to physical scores of interest.

Figure 12 illustrates the setup of volume separation for geometry splitting in the improved Monte Carlo code used in this study. First the whole tissue was divided into several volumes. The volumes closer to the center of the detector fiber were assigned greater importance values because photons in these volumes are more likely to be detected. The actual radial distances used to separate different volumes for the angled probe with a separation of 300 \( \mu \)m were 0, 0.005, 0.01, 0.015, 0.02, 0.025, 0.04, and 50 cm, which formed a series of hemispheres with increasing radii.

The corresponding importance values were 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015 625. Similarly, the radial distances separating different volumes for the flat-tip probe with a separation of 1500 \( \mu \)m were 0, 0.02, 0.04, 0.06, 0.08, 0.1, and 50 cm. The corresponding importance values were 1, 0.5, 0.25, 0.125, 0.0625, and 0.031 25. When a photon crosses from a volume with a smaller importance value to a volume with a larger importance value, it will be split to multiple photons according to the ratio of the two importance values for the adjacent volumes. If a photon crosses in the opposite direction, it will be rouletted. The roulette probability was determined by the ratio of the two important values as well.

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References

2. N. Ramanujam, “Fluorescence spectroscopy in vivo,” in Ency-

![Fig. 12. Schematic of the geometry splitting setup in the improved Monte Carlo code. The arrows indicate the direction of light. The half-circle arcs represent hemispherical volumes with the displayed numbers denoting different importance values. It should be pointed out that the radii of the arcs were not drawn to scale.](image)


