Noncontact imaging of absorption and scattering in layered tissue using spatially modulated structured light

Jessie R. Weber,1 David J. Cuccia,2 Anthony J. Durkin,1 and Bruce J. Tromberg1,a)
1Laser Microbeam and Medical Program (LAMMP), Beckman Laser Institute and Medical Clinic, University of California, 1002 Health Sciences Rd., Irvine, California 92612, USA
2Modulated Imaging Inc., 1002 Health Sciences Rd., Irvine, California 92612, USA

(Received 1 May 2008; accepted 23 December 2008; published online 19 May 2009)

Sinusoidal patterns of spatially modulated near-infrared (650 nm) structured light are used to interrogate multilayer phantoms and tissue. Diffuse reflectance is imaged at multiple spatial frequencies from 0–0.3 mm−1. ac and dc components of the image are fit to a two layer model formulated from the diffusion approximation to the Boltzmann transport equation. The two-layer model depends on optical properties (absorption, μa, and reduced scattering, μs') in each layer and on top layer thickness (d). Layered tissue phantoms with variable optical properties in each layer (μa=0.006–0.034 mm−1 and μs'=0.89–1.45 mm−1) were constructed to test the accuracy of the model. Constraining top layer thickness to within 25% of the correct value in a four-parameter fit results in recovery of upper layer optical properties with average accuracies of ±2% for top layer μa' and ±17% for top layer μa. Bottom layer μa can then be recovered to an average accuracy of ±25% with two parameter fits. Average accuracies of top and bottom layer absorption can further be improved to 12% and 18%, respectively, by fitting for each alone. Bottom layer scattering and top layer thickness do not vary significantly from initial guesses because of poor sensitivity to these parameters in frequency dependent reflectance data. Measurements of in vivo volar forearm optical properties at 650 nm produced spatially varying skin (d=2 mm) optical property maps that range from 0.025–0.045 and 1.7–2 mm−1 for upper layer μa and μs' and 0.005–0.015 and 0.5–3 mm−1 for lower layer μa and μs', respectively. These preliminary results suggest that spatial modulation of the source provides sufficient depth sensitivity to allow noncontact mapping and quantification of layered tissue optical properties using a wide-field, noncontact approach. © 2009 American Institute of Physics. [DOI: 10.1063/1.3116135]

I. INTRODUCTION

Noninvasive characterization of tissue using diffuse light is a well-established approach for reporting endogenous physiological contrast based on tissue optical properties.1–5 The tissue optical properties considered here are absorption and reduced scattering, μa and μs', respectively. Measuring the level and distribution of absorbers allows us to study tissue physiologic function, such as the concentration of oxygen and deoxyhemoglobin, lipids, and water.6,7 The reduced scattering parameter is correlated with the size, density, and distribution of tissue scattering components and hence tissue structure. Scattering differences occur between types of tissue and as a result of structural changes in cells, vasculature, and extracellular matrix associated with pathologies.8,9

Current methods of determining tissue optical properties often employ the diffusion approximation to the Boltzmann transport equation.10–12 In this approximation, photons are modeled as diffusive particles moving through a highly scattering, macroscopically isotropic medium, driven by a gradient of photon density from the source. This involves the assumption that bulk tissue is spatially homogeneous. Although this assumption has proven useful in a number of situations, tissue is rarely homogeneous. A spatial heterogeneity common to tissue is the presence of distinct layers. Layered systems in the body include epithelial structures such as skin and the aero-digestive tract. The use of homogeneous models to calculate optical properties of layered structures may result in loss of important physiological information from one or both layers.

In order to separate optical properties from different layers, a number of groups have developed multilayer diffusion-based models13–18 While these techniques provide information about the inhomogeneity of the layers in the axial direction, most do not provide transverse mapping capabilities. This is because they rely on point measurements, which characterize some volume of tissue between a source and detector (or a few sources and detectors) that are in contact with the tissue, usually in a probe-based geometry. By taking multiple point measurements, sparse mapping can be accomplished. A technique that could easily map all of the transverse spatial locations and distinguish layers in a single measurement would clearly be advantageous.

Here we use spatially modulated plane wave projections and charge-coupled device (CCD) detection in a noncontact geometry to provide maps of tissue optical properties. We have adapted a planar photon density wave (PPDW) model for multilayered turbid media to fit the diffuse reflectance into five parameters: optical properties of each layer (μa1, μs1', and μa2, μs2') and top layer thickness (d). The objectives
II. THEORY AND MODEL

We have previously described solutions to the diffusion equation for multilayered systems using PPDWs. The structure and geometry of the present measurement is based on this work and depicted in Fig. 1.

Spatially modulated planar waves are projected onto tissue or a tissue phantom. The tissue or phantom is assumed to have a layered structure with a top layer of thickness, $d$, and some optical absorption coefficient ($\mu_a$, $\mu_s$) and reduced scattering coefficient ($\mu_s'$, $\mu_s''$) in each layer. Here we make two modifications to the previous model. First, since we are modulating the source in spatial and not temporal frequency, we utilize the time independent diffusion equation,

$$\nabla^2 \varphi - 3 \mu_a \mu_s \varphi = -3 \mu_a q, \tag{1}$$

where $\mu_s = \mu_s' + \mu_s''$, $\varphi$ is the fluence rate and $q$ is the source term. Since the fluence rate only varies with depth ($z$) at each pixel detection point on the CCD camera, this reduces to a one dimensional expression,

$$\frac{d^2}{dz^2} \varphi(z) - 3 \mu_a \mu_s \varphi(z) = -3 \mu_a q. \tag{2}$$

Light within the medium decays exponentially, such that for two layers, the source terms for each layer are

$$q_1 = P_o \mu_s' \exp(-\mu_s' z), 0 < z \leq d \tag{3}$$

$$q_2 = P_o \mu_s'' \exp(-\mu_s'' (z-d)) \exp(-\mu_s z), z > d. \tag{4}$$

In Eqs. (3) and (4), $P_o$ is the incident optical power, $d$ is the top layer thickness, subscript 1 refers to the upper layer, subscript 2 refers to the lower layer, and $z$ is the axial dimension (such that $z=0$ refers to the surface of the medium and $z=d$ refers to the depth between the two layers). We then solve for the fluence rate in each layer,

$$\varphi_1 = \frac{P_o \delta_{\text{exc},1} \mu_s'}{3 \mu_{\text{tr},1} (1 - \delta_{\text{exc},1} \mu_{\text{tr},1})} e^{-\mu_s' z} + A_1 e^{-\mu_s z}, 0 < z \leq d \tag{5}$$

$$\varphi_2 = \frac{P_o \delta_{\text{exc},2} \mu_s''}{3 \mu_{\text{tr},2} (1 - \delta_{\text{exc},2} \mu_{\text{tr},2})} e^{-\mu_s'' z} + A_2 e^{-\mu_s z}, z > d \tag{6}$$

$$\mu_{\text{eff}} = \frac{1}{\delta_{\text{eff}}} = \sqrt[3]{3 \mu_a \mu_s}, \tag{7}$$

where $\delta_{\text{eff}}$ is the effective penetration depth.

To adapt this model for a sinusoidally varying source, a new source function, $S$, is substituted for $q$ in Eq. (1) above,

$$S = S_0 \left( \frac{1}{2} \cos(2 \pi f_x x + \alpha) + \frac{1}{2} \right), \tag{8}$$

where $f_x$ is the spatial frequency of the sinusoids and $\alpha$ is the spatial phase. Plugging this sinusoidal source into the steady state diffusion equation [Eq. (1)] leads to a new expression,

$$\nabla^2 \varphi_{AC} - \mu_{\text{eff}}^2 \varphi_{AC} = -3 \mu_a S_0, \tag{9}$$

where $AC$ refers to the spatial frequency dependent fluence rate and the resulting $\mu_{\text{eff}}$ (called $\mu_{\text{eff}}^*$ and penetration depth ($\delta_{\text{eff}}^*$) are dependent on the spatial frequency,

$$\mu_{\text{eff}}^* = \frac{1}{\delta_{\text{eff}}^*} = \sqrt[3]{3 \mu_a \mu_s + (2 \pi f_x)^2}. \tag{10}$$

Plugging the new $\mu_{\text{eff}}^*$ and $\delta_{\text{eff}}^*$ [Eq. (10)] into the solutions for the fluence rates in each layer [Eqs. (5) and (6)], we arrive at

$$\varphi_1 = \frac{P_o \delta_{\text{exc},1} \mu_s'}{3 \mu_{\text{tr},1} (1 - \delta_{\text{exc},1} \mu_{\text{tr},1})} e^{-\mu_s' z} + A_1 e^{-\mu_s z} + A_2 e^{\mu_s z}, 0 < z \leq d \tag{11}$$

$$\varphi_2 = \frac{P_o \delta_{\text{exc},2} \mu_s''}{3 \mu_{\text{tr},2} (1 - \delta_{\text{exc},2} \mu_{\text{tr},2})} e^{-\mu_s'' z} + A_2 e^{-\mu_s z} + A_3 e^{\mu_s z}, z > d, \tag{12}$$

where $A_1$, $A_2$, and $A_3$.

Finally, the measured value, diffuse reflectance ($R_d$) is

$$R_d = \frac{j_1|z=d| - j_2|z=d|}{P_0} = \frac{1 - R_{\text{eff}}}{2(1 + R_{\text{eff}})} \delta_{\text{eff}}(|\mu_s'| A_1 + A_2), \tag{13}$$

where $j$ is the flux, $R_{\text{eff}}$ is the effective reflection coefficient ($R_{\text{eff}}=0.493$ for tissue). The constants ($A_1$, $A_2$, $A_3$) can be determined by using the following boundary conditions:

$$\varphi_1|_{z=d} = \varphi_2|_{z=d} \tag{14}$$

$$j_1|_{z=d} = j_2|_{z=d} \tag{15}$$

where condition (14) states that the fluence and flux are continuous across the boundary between layers and condition (15) states that the flux just below surface is related to the fluence just below the surface.

In order to obtain the reflectance at each pixel of the image, each spatial frequency is projected at three phase angles, 0°, 120°, and 240°. These phases are then demodulated using the equation: Reflectance ($f$) = $(2^{1/2}/3)[(A - B)^2 + (B - C)^2 + (C - A)^2]^{1/2}$ where $A$, $B$, and $C$ are the reflectance
images for the three phase projections, as described by Cuccia et al. The resulting reflectance value at each pixel is the AC, or frequency-dependent, reflectance at that pixel, allowing each pixel to be handled independently.

III. MATERIALS AND EXPERIMENTAL METHODS

A. Instrumentation

Modulated imaging (MI) is a wide-field, noncontact method that uses sinusoidal patterns of structured light to map tissue absorption (\(\mu_a\)) and scattering (\(\mu_s\)) properties. The current system is shown in Fig. 2. The light source is a 250 W, power adjustable tungsten halogen lamp (Newport Oriel) with a controller unit to stabilize the power output of the bulb over time. The filament of the bulb is homogenized and imaged onto a digital micromirror device (DMD). The DMD, 1024 \(\times\) 768 binary mirrors, based on the DLP™ technology (Texas Instruments), produces the spatially modulated patterns. The mirrors reflect light from the light source into the projection optics and then onto the sample. From the sample, collection lenses direct light into a CCD camera. The Nuance camera (Cambridge Research & Instrumentation, Inc.) employs a liquid crystal tunable filter, which can be computer controlled to sequentially pass wavelength bands in the 650–1000 nm range with spectral resolution of 10 nm and a pixel array of 1392 \(\times\) 1040 pixels. The field of view using this camera is variable and dependent on the projection and collection lenses chosen, typically on the order of several centimeters. Crossed linear polarizers are used to eliminate specular reflection from the images (one is placed after the projector and the other is placed before the camera, with the sample between).

B. Phantoms

Two-layer liquid phantoms with variable optical properties were made from Intralipid (Intralipid 20%, Pharmacia, Inc., Clayton, NC) and water-soluble nigrosin (Sigma-Aldrich Inc., St. Louis, MO) to simulate tissue scattering and absorption, respectively. The bottom layer consisted of a large, open-top container (20 cm diameter, 6 cm deep). A 50 \(\mu\)m thick, optically transparent layer of mylar (Reynolds Film, Reynolds Metals Co., Richmond, VA) was placed over the bottom layer, creating a well for a top layer to sit. This film has previously been shown to have no effect on the optical measurement of a two-layer system.

The optical properties of each layer were individually varied and measured, while all other parameters were held constant. While varying the optical properties, the top layer thickness was held constant at 4 mm. To increase absorption in a given layer, stock nigrosin (500 mg/L) was added. Similarly, Intralipid was added to increase scattering in each layer. The expected absorption and scattering were calculated from the total content of nigrosin, Intralipid, and water present in each case. When varying the bottom layer, top layer optical properties were lowered slightly to simulate an effectively thinner top layer. A 2.5 mm layer of \(\mu_a\) = 0.018/mm and \(\mu_s\) = 1.11/mm at 650 nm was simulated by matching \(\mu_a\) \(\times\) layer thickness, resulting in a 4 mm layer with \(\mu_a\) = 0.011/mm and \(\mu_s\) = 0.7/mm. Table I shows the values of optical properties measured in each layer and the values of constant optical properties for the parameters that were held constant.

Diffuse reflectance measurements of the two-layer phantoms were performed using six spatial frequencies ranging from 0–0.3 mm\(^{-1}\) at 650 nm. Measurement of a homogeneous liquid phantom was performed for system calibration (\(\mu_a\) = 0.018/mm and \(\mu_s\) = 1.11/mm at 650 nm).

C. In vivo measurement

A single measurement of volar forearm (Caucasian) was taken as an in vivo example of a layered system consisting of skin over subcutaneous tissue (in vivo measurements were approved by the Institutional Review Board of the University of California, Irvine). The measurements were made at 650 nm using six spatial frequencies between 0–3 mm\(^{-1}\). The field of view of the measurement was 20 by 24 mm. Since the main intent of this study is to understand our ability to recover optical properties in layered structures, we selected an area of forearm without spatial heterogeneity in the transverse direction. We did not wish to complicate the measurement by having to understand transverse resolution, which we have previously shown degrades with depth and may be about millimeter scale at a 1–2 mm depth. As our transverse resolution studies progress, we anticipate combining these with our multilayer model.

D. Data analysis

The processing of the data is outlined in Fig. 3. First, a set of frequencies is projected onto the tissue, with each frequency projected at three phase angles, 0°, 120°, and 240°.

### Table I. Properties of the two-layer phantoms.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range when varied (mm(^{-1}))</th>
<th>Constant value (mm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu_a)</td>
<td>0.006–0.034</td>
<td>0.018 (0.011(^a))</td>
</tr>
<tr>
<td>(\mu_s)</td>
<td>0.89–1.45</td>
<td>1.11 (0.7)(^a)</td>
</tr>
<tr>
<td>(\mu_a)</td>
<td>0.006–0.034</td>
<td>0.018</td>
</tr>
<tr>
<td>(\mu_s)</td>
<td>0.89–1.45</td>
<td>1.11</td>
</tr>
<tr>
<td>(d)</td>
<td>N/A</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^a\)Constant value for lower layer variations.
These phases are then demodulated using the equation: Reflectance \( f=\left(2^{1/2}\right) \left[(A-B)^2+(B-C)^2+(C-A)^2\right]^{1/2} \) where \( A, B, \) and \( C \) are the reflectance images for the three phase projections.\(^{22}\) Once the frequencies are demodulated, each image is calibrated to a phantom with known optical properties using the same frequency projections. This calibration corrects for inhomogeneity in the light source and system. This yields a wide-field diffuse reflectance image for each spatial frequency. Finally, using a minimum of two spatial frequencies, the diffuse reflectance for each wavelength and each pixel is fit using a nonlinear least-squares fit to recover absorption and reduced scattering, \( \mu_s \) and \( \mu'_s \), respectively. Fitting at each available pixel results in absorption and reduced scattering maps.

To demonstrate the problem of using a homogeneous model to fit a layered system, diffuse reflectance data from a two-layered phantom (in which the absorption of the two layers was different) were fit to our standard homogeneous model. The homogeneous fit values for absorption and reduced scattering were compared to the known values in each layer of the phantoms.

Next, the diffuse reflectance data from the two-layered phantoms at all six spatial frequencies (0–0.3 mm\(^{-1}\)) were fit to the two layer model. Four types of fits differing in the number of constrained parameters were performed. First, all five variables, \((\mu_{s1}, \mu'_{s1}, \mu_{s2}, \mu'_{s2} \text{ and } d)\) were fit at once (five-variable fit). Next, all optical properties were fit while top layer thickness was held constant at the correct value (four-variable fit). Optical properties in a single layer were varied, either top or bottom, depending on which layer had a changing variable, while the other layer’s optical properties and the top layer thickness were held constant (two-variable fit). Finally, single-variable fits were performed, in which only the parameter varied was fit, while all others were held constant at the correct values.

TABLE II. Accuracies of fit types, reported in average accuracy over the range of the variation of the optical property.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Five-variable fit</th>
<th>Four-variable fit</th>
<th>Two-variable fit</th>
<th>One-variable fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_{s1} )</td>
<td>23.5%</td>
<td>17.2%</td>
<td>22.4%</td>
<td>11.8%</td>
</tr>
<tr>
<td>( \mu_{s2} )</td>
<td>83.6%</td>
<td>99.5%</td>
<td>25.2%</td>
<td>18.14%</td>
</tr>
<tr>
<td>( \mu'_{s1} )</td>
<td>1.2%</td>
<td>1.2%</td>
<td>1.6%</td>
<td>3.8%</td>
</tr>
<tr>
<td>( \mu'_{s2} )</td>
<td>21.3%(^a)</td>
<td>21.2%(^a)</td>
<td>61.4%(^a)</td>
<td>26.5%(^a)</td>
</tr>
<tr>
<td>( d )</td>
<td>25%(^b)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

\(^a\) stays within 5% of the initial guess of 0.9 mm\(^{-1}\) for all fits.
\(^b\) stays to within 5% of the initial guess of 5 mm for all five-variable fits.

Several initial guess schemes were tested to assess the impact of initial guess on fitting accuracy. In order to partially constrain the problem, bounds for absorption and scattering were set at 0.005–1 and 0–5 mm\(^{-1}\), respectively. These constraints were used as the standard fitting method throughout the data analysis. We observed that bottom layer reduced scattering and top layer thickness, when fit, did not move more than a few percent from the initial guess until the initial guess was more than 100% from the actual value. However, fit values of these quantities within 25% of their true values did not compromise accuracy of the other free parameters. We note here that overestimates in top layer thickness tend to result in smaller top layer absorption errors than underestimates in top layer thickness, while top layer scattering accuracy remains robust regardless of the constrained value for top layer thickness. Very little sensitivity to changes in bottom layer scattering in the measurement leads to very little impact of a wrong constrained value. When deviating up to 300% from the correct value, all fit parameters except top layer absorption maintain levels of accuracy reported in Table II. Top layer absorption degrades after a 25% underestimate of bottom layer scattering, but accuracy does not degrade with an overestimate of bottom layer scattering of up to 300%.

Accuracies from the different types of fits from the two-layer liquid phantoms were used to inform the fitting of the \( \text{in vivo} \) data. Top layer thickness was held constant at 2 mm in all of the \( \text{in vivo} \) fitting.

IV. RESULTS AND DISCUSSION

A. Two-layer phantoms

Figure 4 shows the frequency dependent diffuse reflectance data for each of the optical property variations performed. Each plot shows the five different optical property values over the varied range. These graphs demonstrate the sensitivity of the measurement at each spatial frequency to changes in optical properties within each layer. In all of the plots, frequency dependent decay is observed. This is expected, due to the depth-dependent damping of the fluence at higher spatial frequencies, as predicted by Eqs. (9)–(11). The plots in Fig. 4 show that increases in absorption decrease diffuse reflectance, while increases in scattering lead to increased reflectance. For variations in upper layer optical properties, the best absorption contrast is observed at the lowest spatial frequencies [Fig. 4(a)], while reduced scatter-
ing contrast is apparent across the spatial frequency range [Fig. 4(b)]. Overall, the greatest measurement sensitivity is to top layer scattering variations [Fig. 4(b)], while sensitivity to variations in bottom layer scattering are weakest [Fig. 4(d)]. These differential sensitivities between layers can be explained in the context of the frequency dependent penetration depth [Eq. (9)]. As spatial frequency increases, the average penetration depth is reduced. For dc illumination (spatial frequency = 0 mm$^{-1}$) the deepest average penetration depth is achieved.

Overall, bottom layer contrast is less than that of the top layer, due to the fact that light must travel through the top layer twice before it is collected in the measurement. For variations in bottom layer absorption [Fig. 4(c)], there is a small amount of contrast at the zero spatial frequency, but it appears that the best absorption contrast is observed at higher spatial frequencies. The model only predicts contrast for lower layer absorption at the zero spatial frequency. The apparent contrast extending to higher spatial frequencies is still very small and could be attributed to phantom fabrication error or calibration effects. Ultimately, increasing spatial modulation frequency substantially above the reciprocal of the layer thickness is expected to confine light penetration to the upper layer (~1.5 mm). With thinner layers and/or lower scattering, we expect that sensitivity to $\mu_s'$ might improve.

Results of the different two-layer fitting paradigms are shown in graphs of fit versus expected optical properties (Figs. 5 and 6). The expected optical properties are those calculated from the concentrations of nigrosin and Intralipid. Each graph shows the results of various data fitting strategies spanning from five free parameter fits, to constraining four variables and solving for a single free parameter. Accuracies for the different fits are summarized in Table II.

Figure 5 shows fits for top layer optical properties. In Fig. 5(a) we can see fairly good performance for fitting top layer absorption in all of the fits. The accuracy improves as we constrain more variables, with the best results when only top layer absorption is allowed to vary. Standard deviation across this range of fit values during all fits was always within 10% of the mean values. Figure 5(b) shows excellent performance in recovering top layer reduced scattering for all types of fits. We note that the greatest error appears in the one-variable fit for top layer scattering, but this error is small (less than 6%) and could be the result of systematic error in phantom fabrication. Standard deviations in all fits were always within 1% of the mean fit values.

Figure 6 shows the two- and one-variable bottom layer absorption fits. The five- and four-variable fits recovered va-
ues for absorption within an average of about 100% error. Both two- and one-variable fits greatly improve the accuracy, with a general trend of slightly underestimating absorption at higher values. The standard deviations for lower layer absorption and scattering for all fits across the ranges of values were within 10% and 1% from the mean fit values, respectively.

It was found that when fitting for bottom layer scattering, even in a single variable fit, the $\mu_s$ value did not often deviate from the initial guess. This is not surprising since, in Fig. 4(b), we saw that the measurement is weakly sensitive across the spatial frequency range when bottom layer scattering is varied. Although the overall impact of a change in bottom layer scattering on diffuse reflectance is small, there may be potential to resolve these changes perhaps by using other light propagation models.

These results show that we can recover top layer optical properties and bottom layer absorption. Since a five-variable fit does not allow us to accurately recover all of the properties, we propose a stepwise algorithm. First, top layer thickness must be known to within $\pm 25\%$ of the true value. This could be accomplished prior to the measurement using ultrasound, or using historically published values for skin thickness for various locations on the body. Next, a four-variable fit can be used to obtain top layer reduced scattering and top layer absorption. Finally, top layer optical properties can be used in a two-variable or one-variable fit to determine bottom layer absorption.

Diffuse reflectance values from two-layered phantoms were also fit to a homogeneous model. In general, $\mu_a$ and $\mu'_s$ fall nearly halfway between the actual values for each layer. Table III shows a sample two-layer phantom with different optical properties in each layer, with the expected optical properties in each layer, the two-layer fit values, and the homogeneous fit values. Deviations between true values and model fits vary for different combinations of $\mu_a$ and $\mu'_s$ in each layer. The highest impact is on the recovery of $\mu_s$.

### B. In vivo volar forearm

Data from a single volar forearm measurement (Caucasian) were fit with the two-layer model and compared with previously reported values for optical properties in each layer and top layer thickness. The top layer was considered to be skin and the bottom layer subcutaneous tissue. Top layer thickness was held constant at 2 mm. The average values for upper and lower layer properties from four-variable fits (all optical properties varied, thickness held constant) and one-variable fits, respectively, appear in Table IV with previously published values for comparison. Absorption and scattering in the bottom layer are expected to be lower than top layer values due to the differential composition of the tissue layers. Skin is composed of cells, extracellular matrix, and blood, while subcutaneous tissue is mostly lipid. Cells and extracellular matrix are expected to have higher scattering than lipid. Blood is the major tissue chromophore at 650 nm and we expect to see more blood in skin than subcutaneous tissue. The fit values for the upper layer optical properties fall close to the published values for skin (epidermis and dermis). When a four-variable fit is used to find bottom layer properties, bottom layer absorption falls to the lower bound of the fitting constraint (0.005 mm$^{-1}$) and bottom layer scattering returns values that nearly always fall on the initial guess (1.44 mm$^{-1}$). Overall, the four-variable fit can be relied on

---

**FIG. 5.** (Color) Top layer optical property fits. In (a) fit vs expected top layer absorption is shown for different fitting constraints. For a cropped and binned image with $n=176$ pixels, standard deviation among all fits and values was always within 10% of the mean. In (b) fit vs expected top layer scattering is shown. For a cropped and binned image with $n=176$ pixels, standard deviation among all fits and values was always within 1% of the mean.

**FIG. 6.** (Color) Bottom layer optical property fits. (a) Fit vs expected bottom layer absorption is shown for different levels of fitting constraints. For a cropped and binned image with $n=176$ pixels, standard deviation among all fits and values was always within 1% of the mean.
TABLE III. Two-layer and homogeneous fit for a two-layer liquid phantom with different optical properties in each layer, compared with expected values.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Two layer fit (mm⁻¹)</th>
<th>Expected values (mm⁻¹)</th>
<th>Homogeneous fit values (mm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{s1}$</td>
<td>0.01 ± 0.0004 (four-variable fit)</td>
<td>0.011</td>
<td>$\mu_a$ 0.014 ± 0.0004</td>
</tr>
<tr>
<td>$\mu_{s1}'$</td>
<td>0.71 ± 0.0037 (four-variable fit)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>$\mu_{s2}$</td>
<td>0.019 ± 0.0014 (one-variable fit)</td>
<td>0.028</td>
<td>$\mu_a$ 0.72 ± 0.0037</td>
</tr>
<tr>
<td>$\mu_{s2}'$</td>
<td>0.84 ± 0.003 (one-variable fit)</td>
<td>1.11</td>
<td></td>
</tr>
</tbody>
</table>

for upper layer optical properties. Next, the values for top layer optical properties and bottom layer scattering from the four-variable fit were used to constrain one-variable fits for bottom layer absorption, with the results appearing in Table IV. The resulting average value for bottom layer absorption was 0.006 mm⁻¹, much closer to the published values. This agrees with the phantom data, in which accuracy was improved by 65%–70% by using a one-variable fit compared with a five-variable or four-variable fit. Finally, the value for lower layer absorption was used to constrain a one-variable fit for bottom layer scattering. Note again that the homogeneous fit (right column in Table IV) provides optical properties that are some average of the optical properties of the two layers.

The unprocessed diffuse reflectance image with planar illumination at 650 nm appears in Fig. 7. Maps of the fit top layer optical properties (four-variable fit) appear in Fig. 8. Highly absorbing hairs are visible in the absorption map, but not in the reduced scattering map. The surface creases in the skin appear more defined in the reduced scattering map than in the absorption map. Overall, the range of top layer absorption and reduced scattering appearing in the maps agree with the published ranges of optical properties for skin. The histograms in Fig. 8 show Gaussian distributions of values for the 2.09×10⁶ pixels shown (based on 2×2 binning and a cropped region of interest from the original images). This type of analysis allows us to evaluate the statistical distribution of optical properties in any region of interest. Small standard deviations from the mean values (full width at half maximum of ~0.01 mm⁻¹ for $\mu_{s1}$, ~0.1 mm⁻¹ for $\mu_{s1}'$) are observed.

Figure 9 shows the lower layer optical property maps resulting from four-variable fits. The histograms below show the distribution of pixel values for the 2.09×10⁶ pixels shown (from a cropped region of interest). In Fig. 9(a), bottom layer absorption, the most noticeable spatial feature is hair. The appearance of hair in the lower layer is most likely an artifact resulting from the inability of the diffusion-based model to accurately image through highly absorbing structures. From the histogram, we can see that most of the fit values fall to the lower bound of the fit at 0.005 mm⁻¹. A distribution of reasonable values exists between the lower bound and the high absorption value for the hair artifact of ~0.015 mm⁻¹. These values are lower than the values for top layer absorption, due to the lower amount of blood expected in the subcutaneous tissue in the bottom layer compared with the skin in the upper layer, as previously discussed. In the lower layer reduced scattering histogram [Fig. 9(b)], we can see that most of the values fall exactly on the initial guess of 1.44 mm⁻¹ indicating that the fit does not significantly deviate from the initial guess. We note that most pixels in Fig. 9(b) (73% of the pixels) have the value of 1.44 mm⁻¹ and that the distribution appears to be singular at that value with tiny side bands falling higher and lower.
These side bands in the distribution are probably a consequence of the clustering around the initial guess and the difficulty the algorithm has in predicting values in bottom layer scattering. Thus, most points fall to the local minimum at the initial guess.

V. CONCLUSIONS

This work is a first demonstration that spatially modulated structured light can be used to map optical properties in a two-layer system. Tissue phantom experiments confirm that three of five free parameters: top layer $\mu_a$ and $\mu'_s$ and bottom layer $\mu_a$ can be recovered with reasonable accuracy using a sequential fitting strategy. However, recovery of bottom layer scattering was difficult to achieve due to the relatively poor sensitivity of the measurement to variations in $\mu'_s$. This is expected to improve for thinner layers and lower values of $\mu'_s$ where $1/\mu'_s \sim d$. A multispectral measurement that allows the use of a power-law fit for reduced scattering may also improve estimates of $\mu'_s$.

We did not perform a systematic examination of upper layer thickness because, in the case of five variable fits, values for $d$ did not deviate significantly from initial guesses. Clearly, for the values of $d \times \mu'_s$ explored in this paper, spatial frequency modulation was relatively insensitive to $d$. As a result, we constrained upper layer thickness to within 25% of the correct value to optimize recovery of $\mu_a$ and $\mu'_s$. Future work should examine this issue in greater detail so that proper selection of spatial frequencies can be made for a given measurement system. In addition, we previously reported an approach using temporal modulation with the ability to recover $d$ to within 1 mm for layers up to 10 mm. This approach combining spatial and temporal modulation may enhance performance in multilayered systems.

Overall, this preliminary work demonstrates the potential for accurate mapping of absorption and reduced scattering in a 2–4 mm thick upper layer. This spatial frequency “filtering” can constrain the depth sensitivity of near-infrared light which, depending on the precise wavelength, may interrogate a deeper average volume without spatial modulation. This has direct implications for clinical dermatology, allowing for quantification and mapping of absorption and reduced scattering in the skin, while filtering out the contributions from subcutaneous tissue. Expansion of this work has the potential to inform problems involving absorption changes in a lower layer, such as those commonly encountered in small animal model imaging of tumors and neural tissue.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health under Grant Nos. P41-RR01192 (Laser Microbeam and Medical Program: LAMMP) and U54-CA105480 (Network for Translational Research in Optical Imaging: NTRI). Programmatic support by the Beckman Foundation and Military Photomedicine Program, AFOSR Grant No. FA9550–08–1–0384 is gratefully acknowledged. Jessie Weber thanks the ARCS® Foundation, Inc. for their generous support.