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Multi-layer silicone phantoms for the evaluation of quantitative optical techniques in skin imaging

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ABSTRACT

With the development of multilayer models for the analysis of quantitative spectroscopic techniques, there is a need to generate controlled and stable phantoms capable of validating these new models specific to the particular instrument performance and/or probe geometry. Direct applications for these multilayer phantoms include characterization or validation of depth penetration for specific probe geometries or describing layer specific sensitivity of optical instrumentation.

We will present a method of producing interchangeable silicone phantoms that vary in thickness from 90 microns up to several millimeters which can be combined to produce multilayered structures to mimic optical properties of physiologic tissues such as skin. The optical properties of these phantoms are verified through inverse adding-doubling methods and the homogeneous distribution of optical properties will be discussed.

Key words: optical phantom; tissue spectroscopy; layered media; optical properties

INTRODUCTION

Applying diffuse optical and quantitative spectroscopic techniques towards skin imaging and dermatology is not a trivial matter. It is well understood that skin tissue is a highly layered and structured medium. These layers, however, are often significantly less than the characteristic transport length of the source illumination as it interacts with tissue. As a consequence, optical measurements typically interrogate tissues from multiple layers and represent an unknown combination of optical properties from these structures.

While advanced modeling techniques, such as multi-layer Monte Carlo, can simulate the behavior of light transport for layer systems with structures smaller than typical transport lengths, they require assumptions of layer thicknesses and/or optical properties in order to provide any precise inverse solution. These modeling approaches can provide valuable insight into the efficacy and sensitivity of optical techniques related to the challenges of imaging tissues, such as skin, however, they are conducted under idealized conditions and typically cannot account for all instrument and system performance functions that may be present in an actual physical measurement.

Tissue-simulating optical phantoms provide the opportunity to evaluate the performance of optical and spectroscopic instruments under controlled experimental conditions. A thorough review of a variety of materials and methods for producing optical phantoms with known or verifiable properties has been published.¹ In the context of producing layered structures in optical phantoms, technical limitations still exist that prevent them from adequately mimicking the physical dimensions found in skin. Layered phantoms have been produced using liquid media², and solid organic compounds such as gelatin and agar.³ These phantoms have only coarse control over layer thickness and can only produce layers to 1 mm resolution. In skin, the epidermal layer thickness can vary from 60-500 microns, depending on the particular individual and location on the body. Additionally, these organic compounds have a limited shelf life (on the order of days) before they degrade and are not directly verified for the optical properties specific to the layers produced.

The targeted goal of this project is to generate sets of interchangeable sheets of turbid materials with stable, independently verified optical properties. 1) The thicknesses of these layers will reflect physiologically relevant values for all layers of skin. 2) The optical properties of each layer can be directly and independently verified prior to being assembled into a multilayer system. 3) Multiple thicknesses for a given set of optical properties will be produced to cover a physiologically relevant range.

Ultimately we will build multi-layer phantoms for characterization and validation of optical (non-contact and probe) based systems, which can map out a parameter space that represents physiological variance. Additional requirements for these phantoms are that they will be stable and reusable so that they can be used across multiple systems and measurement platforms or to track system performance during the development process.

METHODS AND MATERIALS

To accomplish the specific goals stated above, we chose to use silicone as the base material for our layered tissue simulating phantoms, specifically polydimethylsiloxane (PDMS). This medium has distinct advantages which make it an attractive choice. It has a long shelf life and can maintain the same optical properties over a time frame of months to years, if stored properly. The material is solid when cured, yet remains flexible. This not only makes it easily amenable to both contact and non-contact optical modalities, but also permits each sheet to be stacked upon each other without the use of any intermediary contact gels or air gaps. The refractive index of silicone is 1.43, which is a close approximation of tissue, which is often characterized with an index of 1.4.

There are, however, certain disadvantages to using this material. Silicone is hydrophobic, so any water based absorbing agent, such as organic chromophores, are not easily or effectively incorporated into the material. This limits the selection of optical absorbing agents used, such as alcohol based dyes, or necessitates the use of strong solvents like acetone or xylene.

For this initial investigation, we started with a 3-layer model for skin tissue, namely epidermis, dermis and a subcutaneous fat layer. We chose to work with three distinct absorbing agents these respective layers: freeze-dried coffee, dissolved in methanol, alcohol-soluble nigrosin and india ink. Table 1 describes the specifications for each layer as well as the relative concentrations of absorber and scatterer used to produce the desired optical properties.

Table 1 Targeted physical and optical properties and materials for layer-specific phantoms

Layer	Epidermis	Dermis	Subcutaneous Fat
Thickness range	90-500 micron	1-3 mm	Semi-infinite (~3-4 cm)
Target Absorption @650nm	0.2 mm ⁻¹	0.05 mm ⁻¹	0.015 mm ⁻¹
Absorbing Agent	Coffee (10mg/mL)	Nigrosin (0.05mg/mL)	India Ink (0.2mg/mL)
Target Reduced Scattering @650nm	3.0 mm ⁻¹	2.0 mm ⁻¹	1.5 mm ⁻¹
Scattering Agent	TiO ₂ (2mg/mL)	TiO ₂ (1.45mg/mL)	TiO ₂ (1mg/mL)

The choice of utilizing coffee as the absorbing agent in the epidermal phantom layer was not arbitrary. It has been already mentioned within existing literature that the absorption spectrum from coffee exhibits very similar characteristics to that of melanin.³ Both chromophores lack distinct spectral features, but rather exhibit a strong, power law-like decay from the visible into the near infrared, fig 1. This broad, yet featureless property poses challenges to most spectral decomposition methods, coupled by the fact that the epidermal layer is much thinner than the mean transport length of light used to interrogate tissue. For that reason, modeling the epidermal layer with a similarly ambiguous substitute will have a profound effect on the evaluation of optical and spectroscopic methods

and result highly relevant insight into the performance of these systems in their application toward quantitative skin imaging.

Since our choice of base material is hydrophobic, we had to develop an alternative method for incorporating coffee into the phantom. We found that it is possible to dissolve free-dried instant coffee in methanol. Although only a small portion of the coffee would actually dissolve into suspension, the methanol was sufficient enough as a solvent to break down the freeze-dried crystals into a viscous sludge. It was then possible to incorporate this viscous coffee solution into the silicone and distribute it evenly.

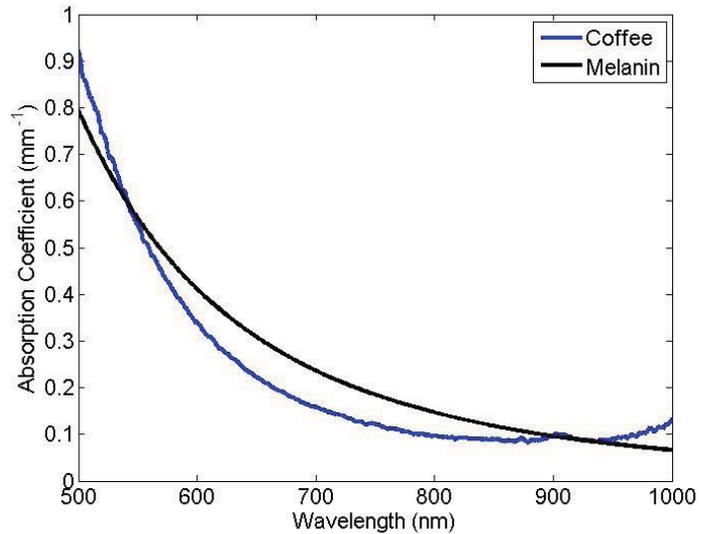


Figure 1 Comparison of Absorption Spectra from Melanin and Coffee

Phantom Fabrication Process

Figure 2 shows the process through which these phantoms are produced. This process has been adopted from Ayers, et. al.⁴ with only a minor modification occurring at the “layer molds” stage of the process. The Ayers process has become standard practice in our lab, since it has developed explicit methodology to address the issues of ensuring even distribution of TiO₂, incorporation of absorbing agents, and removal of air bubbles, introduced by the mixing process, from the uncured phantom material. As in this process, our recipe also uses a 10:1 ratio of silicone to curing agent (Kit P-4, Eager Plastics). We vary the amount of TiO₂ (Ti-602, Atlantic Equipment Engineers) introduced to the curing agent to fit to the desired target reduced scattering for each layer, and introduce the absorbing agent to the silicone to match the expected values identified in Table 1.

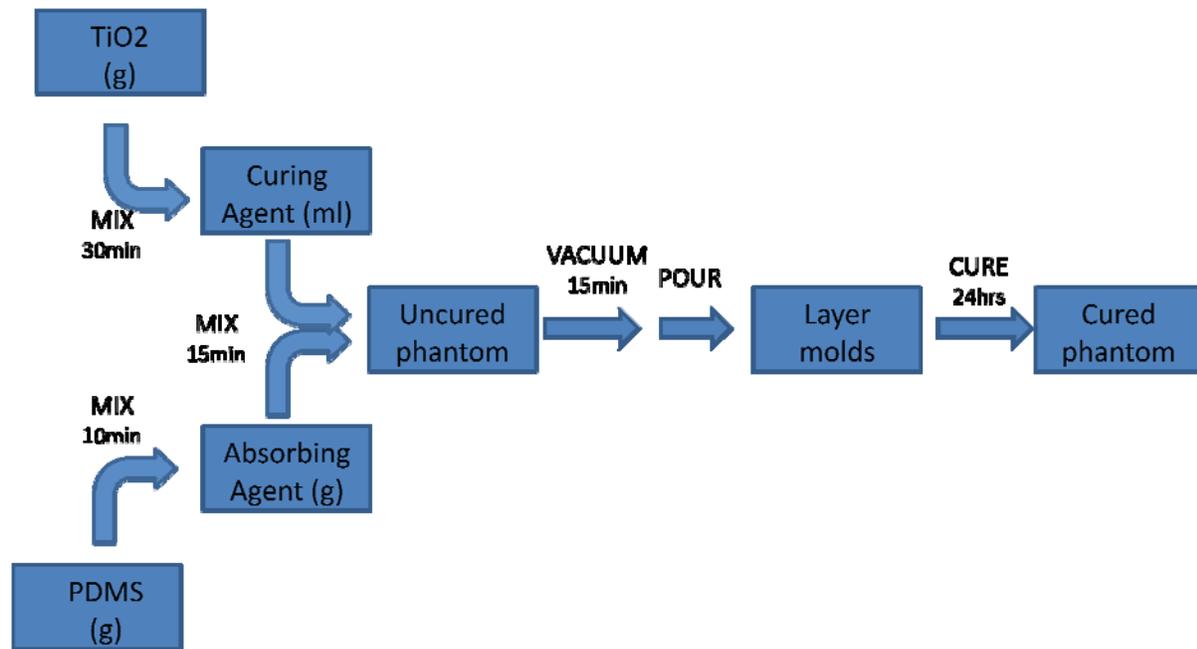


Figure 2 A summary of the primary steps in the phantom making process.

Once the uncured phantom has the residual air bubbles drawn from it in the vacuum chamber, it is poured into a series of custom made wells, Fig. 3. These wells have an outer dimension of 12X12 cm² and an inner well chamber of 8X8 cm². The depth of these wells range from 100 – 600 micron for epidermal layers and 1-3 mm for dermal layers. These well thicknesses were constructed by cutting plastic shim stock material (Color-coded Shim Stock, Artus) into frames and then affixing them to a flat plastic base with epoxy.

Once the phantom material is poured into the center of the well, a painter's edging tool (305 mm Paint Shield, Husky, inc.) was used to draw and spread the material evenly across the area of the well, while the edges of the tool remained in contact with the sides of the well. It is worth noting that this technique for even deposition of materials requires practice. Since the uncured phantom is rather viscous, the resulting deposition thickness is both a function of well depth and speed at which the blade of the painter's edging tool is drawn across the surface. Consistency of speed is critical for even layer thickness, particularly for the thinnest of layers. The ultimate resulting thickness for these layers will not be precisely known until the phantom has cured and can vary tens of microns. For our purposes, it was more critical that the spatial variance of the thickness be minimal. The wells were then placed on a level surface and allowed to cure, uncovered, for 3 days. The total thickness could be determined after the phantom has been fully cured and removed from the wells. Examples of epidermal and dermal phantom are shown in figure 4. These sheets are stored between transparencies to prevent the accumulation of dust.

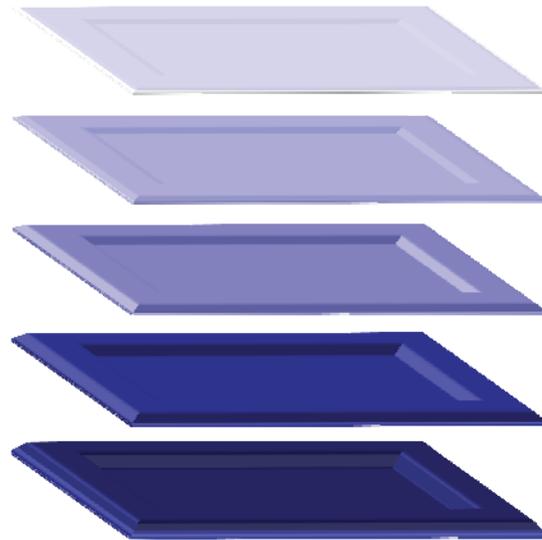


Figure 3 Graphical depiction of multi-layer thickness wells used in the epidermal layer phantom process



Figure 4 Examples of Epidermal phantoms (left) and Dermal phantom (right) produced by this process. The epidermal phantoms, shown here, range from 90 to 500 micron. The dermal example is 1.93 mm thick.

RESULTS

As mentioned in the previous section, these recipes give general expectations for optical properties, but lacks accurate account of ultimate properties produced in a cured phantom. It is therefore necessary to develop appropriate methods to precisely quantify both physical and optical properties for each phantom produced. These

methods not only need to determine the bulk properties of each phantom, but additionally, determine the amount of variation present in each one due to this particular production method.

The thickness of each phantom can be measured directly by calipers. Taking measurements at multiple locations of each sheet can give a gross estimation of any thickness variation that may be present. In our lab, however, we also have access to a high-frequency ultrasound instrument (EPISCAN-I-200, Longport). With this instrument, we can also determine layer thickness and variation with multiple line scans, 1.5 cm in length. Figure 5 shows a side-by-side comparison of human skin tissue and a stacked layer phantom. In these examples, the layer thicknesses for epidermis and dermis are comparable.

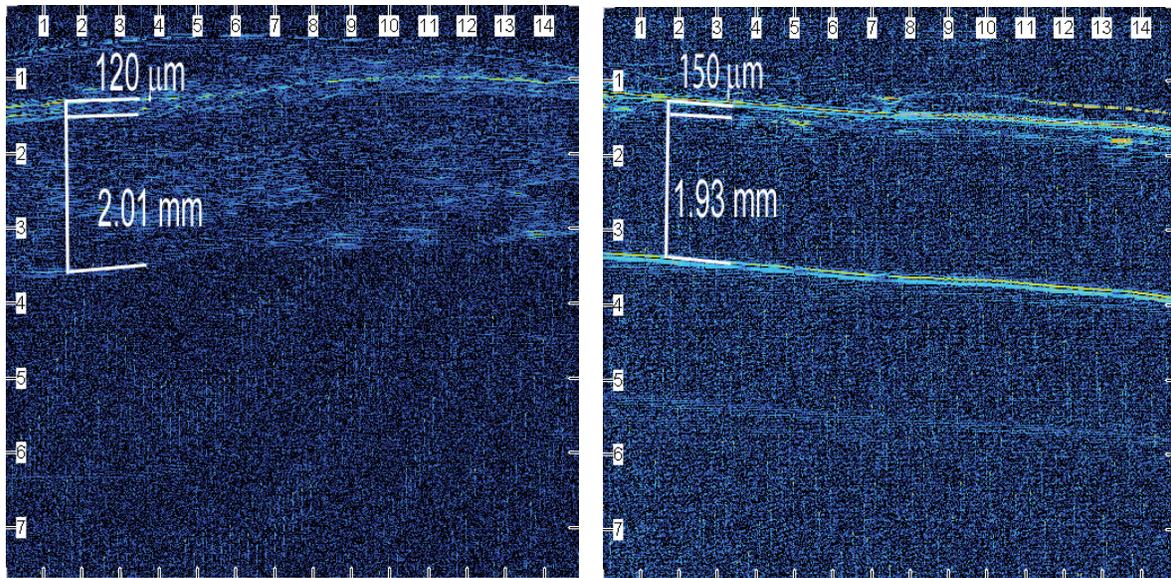


Figure 5 High-frequency ultrasound images of human skin (left) and a stacked layered phantom (right).

The optical properties of each phantom are determined by the Inverse Adding-Doubling (IAD) method. IAD well suited for quantification of thin turbid media.⁵ It is an established, robust technique and processing code is freely available on the internet.⁶ Requisite for this method are calibrated reflectance and transmission spectra for the thin sample in question. To that end, we built a single integrating sphere set up. Using a tungsten-halogen lamp (HL-2000, Ocean Optics) as a broadband source, light from a subtended angle was collimated and an aperture mask was used to create a collimated beam, 3 mm in diameter. This beam is directed to the entrance port of the integrating sphere (4P-GPS-033-SL, LabSphere) and continues through to an exit port 180° from the entrance. 90° from these ports, there is a detector port. At this location, the distal end of a 400 micron fiber collects a portion of the light from the integrating sphere and delivers it to a spectrometer (Oriel, Model #77480). Once the system has been properly

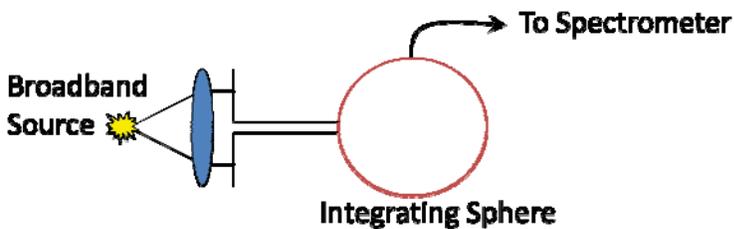


Figure 6 Schematic for single integrating sphere set-up used to collect necessary data for Inverse Adding-Doubling calculation of optical properties.

calibrated, as detailed in Prahl's code documentation, transmission spectra can be collected by placing the sample at the entrance port and a 99% reflectance standard at the exit port. Likewise, reflectance spectra are collected by removing the reflectance standard and placing the sample at the exit port.

With this particular configuration, it is possible to interrogate and determine the optical properties of the layer phantoms over lateral extents that are 3 mm in diameter. To assess the distribution of absorber and scattering agents within each sheet, 5 reflectance and transmission measurement pairs were taken, one from the center of each quadrant of the sheet and a fifth from the center of the entire sheet. Additionally, the measurements across multiple sheets (thicknesses) from a single prepared batch of phantom material, to verify whether there were any significant changes in optical properties as a function of thickness or cure rate.

Phantom Uniformity

Figure 7 shows the calculated absorption and reduced scattering spectra at 5 different locations from the thinnest epidermal layer produced (90 micron) overlaid upon each other. It was anticipated that this particular sheet would produce the highest variability in optical property since it is at the limit to what this particular phantom method can produce. By characterizing the variability of both physical and optical properties as a percent deviation from the mean value across the spectrum (or deviation from the mean thickness for the physical properties), the tolerances for this sheet exceeded initial expectations (Table 2). Figure 8 shows the calculated absorption and reduced scattering spectra from a 1.93 mm thick dermal phantom. Here the tolerances are even tighter, as summarized in Table 2. This is largely due to the fact that the relative increase in thickness reduces the effect of micron scale thickness variations. It is worth noting that the absorption peak near 910 nm is due to the silicone and not due to the nigrosin. It is also present in the epidermal phantoms, but not as noticeable due to the fact that there is considerably relative absorption from the coffee.

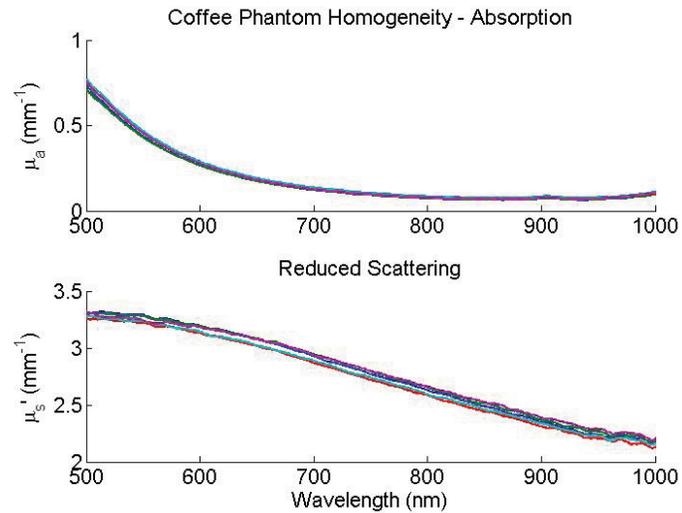


Figure 7 Absorption and reduced scattering spectra calculated from 5 distinct regions of a 90 micron thick epidermal (coffee) layer phantom

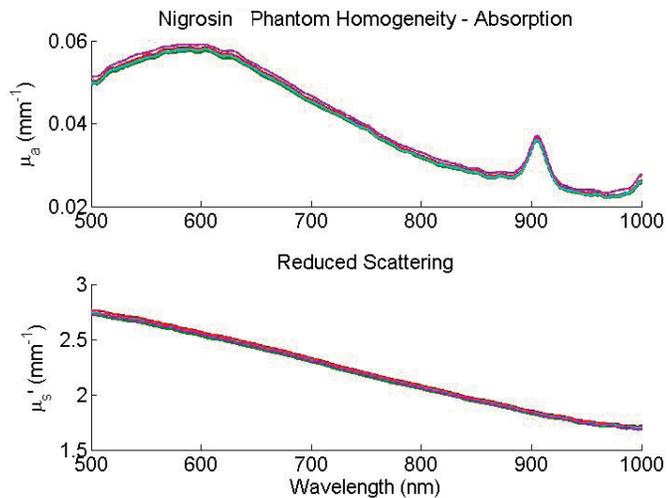


Figure 8 Absorption and reduced scattering spectra calculated from 5 distinct regions of a 1.93 mm thick dermal (nigrosin) layer phantom

Table 2 Tolerances for spatial uniformity for a single sheet

	Thickness	Absorption	Reduced Scattering
Epidermal Layer (90 micron)	+/- 3.9%	+/- 3.5%	+/- 0.9%
Dermal Layer (1.93 mm)	+/- 0.6%	+/- 1.1%	+/- 0.6%

Phantom Consistency

The optical properties of multiple phantoms (multiple thicknesses) were also compared. This comparison was designed to investigate whether curing phantoms at different thicknesses resulted in any significant alteration of scattering properties. It has been noted that as the silicone cures, the total volume may change slightly. Thinner phantoms may be more susceptible to these changes than thicker ones. Also there was concern regarding the consistency of optical property throughout the entire bulk, uncured phantom. In this case, the optical properties from the center of each phantom were compared and tolerances were generated in the same manner as in the uniformity study. Table 3 summarizes these results that indicate that neither the curing process, nor distribution of absorber or scatterer within the uncured phantom plays any substantive role beyond the variations present in a single layer.

Table 3 Consistency of optical property values within a single prepared batch

	Absorption	Reduced Scattering
Epidermal Layers (N=5)	+/- 3.1%	+/- 0.8%
Dermal Layers (N=3)	+/- 1.0%	+/- 0.7%

Subcutaneous Fat Layer phantoms

Unlike the epidermal and dermal layers, the subcutaneous fat layer phantoms are substantially thick and cannot be adequately characterized through IAD methods. As a base layer, they need to be on the order of centimeters thick to be considered optically semi-infinite. Methods detailed elsewhere describe how to determine the optical properties of these phantoms and assess the uniform distribution of absorber and scatterer within these types of phantoms.⁴ Figure 9 shows an example of one such phantom, where the tolerances of absorption and scattering are 0.65% and 0.24%, respectively.

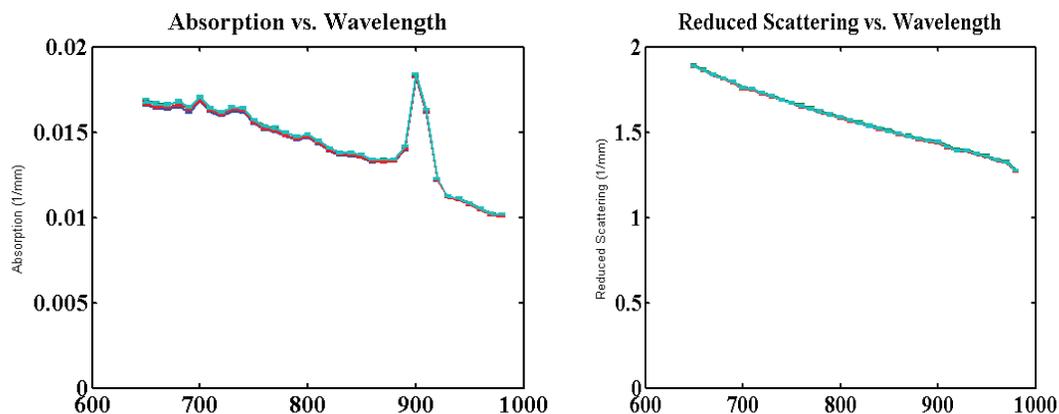


Figure 9 Spatial uniformity of optical properties for a 3 cm thick, subcutaneous fat layer simulating phantom layer. Here, the optical properties were assessed using a spatial frequency domain imaging (SFDI) system, described by Cuccia et. al.⁷

DISCUSSION

From this initial implementation of this layer phantom production method, it has been shown that it is not only feasible to produce layered sheets of tissue simulating silicone phantoms that reflect physiological thicknesses for epidermal, dermal and subcutaneous fat layers, but that these layers can be considered uniform in thickness and

optical property to within a few percent. Given the size of phantom layers produced, they are amenable to the evaluation of wide-field imaging modalities. Given the pliability of the silicone rubber, they are also amenable to contact probe geometries.

Since each sheet is characterized individually, they can be used interchangeably to map out a physiologically relevant parameter space. The uses for this are many-fold: 1) to characterize penetration depth and system performance experimentally, 2) evaluate algorithmic methods for melanin compensation, when quantitative dermal blood perfusion and oxygenation is of interest, 3) characterize resolution and evaluate accuracy of tomographic reconstructions based on depth-sensitive optical techniques, among other uses.

Though the performance of this phantom production method has met with the needs identified in our particular lab, there remain opportunities to improve the tolerances, specifically, with regard to pushing the limits of layer thickness and consistency. Whereas substituting coffee for melanin offers a simple and low cost opportunity to create skin-like epidermal layers, many other dyes, or combinations thereof, have yet to be explored. Of particular interest would be to find a better substitute for hemoglobin within the dermal layers. While this process shows great promise for producing stable and reusable phantoms with a long shelf life, dedicated longitudinal studies on their stability have yet to be performed.

CONCLUSIONS

We have developed a process for producing robust layered phantoms of known optical properties. Although, the method and materials proposed here may be characterized as a bit unrefined, it remains simple, low cost and easily implemented. It has been verified that the distribution of absorber and scattering agents are uniform across each phantom and that the range of layer thicknesses produced are relevant to the physiology present in skin. Using this production method, a wide range of optical properties can be produced, providing a flexible platform to model specific dermatological maladies and evaluate instrument performance within specific application milieus.

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