Optical properties of a simple model of soft biological tissue

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Abstract. Optical phantoms that simulate light propagation processes through soft biological tissue are required to evaluate the performance and calibrate different medical imaging modalities. Liquid lipid based low-cost phantoms were prepared, with cow’s milk as scattering material, red India ink as the absorbing material and demineralized water as the matrix material since it provides a soft medium biologically compatible with the addition of organic molecules. Two experiments were carried out for characterization of this soft tissue model. First, collimated and diffuse transmittance and reflectance spectra were measured using phantoms with milk of different fat content. From the data, dependence between: total extinction coefficient, Kubelka Munk’s ratio, absorption and scattering coefficients on the wavelength were estimated. Second, using collimated transmittance measurements the effect of the phantom components was observed. The absorption peak, around $550 \pm 0.3$ nm, increases as ink was added to milk phantoms; and when lipid concentration was varied, by fixed ink, the scattering grows. The extinction coefficient’s dependence on the wavelength was determined, and fluorescence was observed with a $31 \pm 0.3$ nm Stokes shift. Results confirm the possibility of spectroscopic identification of milk kinds, as well as the feasibility of low cost controllable phantom for preliminary biophotonic studies.

1. Introduction

The study of soft tissue optics is relevant for developing innovative non-invasive diagnostic or therapeutic techniques for pathologies such as cancer and stroke events [1]. To be able to evaluate the performance and calibrate different medical imaging modalities optical phantoms that simulate light propagation processes through soft biological tissue are used [2]. Liquid phantoms are widely used because they can be easily replicated and their construction requires a separating matrix, absorbing and scattering materials. A common choice for the scattering material is Intralipid\textsuperscript{R} or similar lipid based emulsions because they are biologically similar to the bilipid membrane of cells and organelles that cause scattering in tissue, and also because the measurements are highly reproducible since they meet clinical standards and present uniformity between batches. Some dyes of ink are used as stable absorbers to simulate tissue absorption [3]. In our case, as a first approach to the characterization of a simple tissue-like phantom, demineralized water was chosen as matrix material since it provides a soft medium biologically compatible with the addition of organic molecules and looking for a commercial inexpensive option for the absorbing and scattering material, red India ink and cow’s milk were selected.

The main phenomena in light-tissue interaction are absorption and scattering [4] and there are many useful methods to determine the optical constants that describe it [5]. For this study
an indirect method based on the theoretical expressions of the two-flux Kubelka Munk theory was used measuring collimated transmittance \( (T_c) \), and diffuse reflectance \( (R_d) \), transmittance \( (T_d) \) with the double integrating sphere method [6].

2. Materials and methods

For the first experiment three types of milk with different fat contents \( (g/ml) \) were used as the scattering material of the phantoms: whole 3.0% fat milk, lactose-free 1.75% fat milk and fat-free 0.0% fat milk. Samples were prepared in a 250 ml beaker, diluting the milk in demineralised water to obtain lipid concentrations varying from 0.1% to 1.0%, with increments of 0.05%. For the second experiment red India ink was used as the absorbing medium, and whole 3.0% fat milk as the scattering medium. Ink was added to a 200 ml solution with 0.2 ml whole 3.0% fat milk, i.e. with 0.1% lipid concentration using an 50 \( \mu l \) electronic micropipette and the collimated transmittance spectrum was measured for increasing concentrations of ink ranging from 0.005% to 0.2%. Likewise, keeping ink concentration constant at 0.2% with 400 \( \mu l \) diluted in 200 ml of demineralised water, milk was added to the solution with a 5 ml syringe varying lipid concentration from 0.1% to 0.5%.

Spectra \( T_c, R_d \) and \( T_d \) of the phantoms were measured in a broadband wavelength (400 nm - 800 nm) to determine the optical properties of the phantoms materials. The experimental setup for collimated transmittance measurements consisted on a broadband halogen light source (NI-30 Fiber Optic Illuminator, Nikon Instruments Inc.), collimation, focusing and transport optics, CVD plastic cuvette and cuvette holder [7] as shown in Figure 1. A lens-optical fiber system was used to collect the light, which was analyzed and detected by a HR-2000C+ Ocean Optics Spectrometer connected to a computer. For the diffuse measurements a pair of integrating spheres was used [8] as shown in Figure 2 and for fluorescence measurements Ocean Optics LEDs of different wavelengths and lasers of 405 nm and 532 nm were used in the setup shown in Figure 3. The spectra were taken over the VIS wavelength range using SpectraSuite software, three recordings of data for each concentration to reduce error and reference spectra were checked before each measurement.

![Figure 1. Collimated transmittance experimental setup. (a) Block diagram. (b) Setup used.](image-url)

For the estimation of the total extinction coefficient from \( T_c \) we have Beer-Lambert law, Equation (1) [9].

\[
-Ln(T_c) = \sigma_t = (\sigma_a + \sigma_s).
\]

And for the determination of optical constants we used a method based on Kubelka Munk model [10] for diffuse transmission and reflection presented by Van Gemert assuming index of refraction matching [11]. First, KM ratio \( \frac{K}{S} \) was calculated with Equation (2).
\[
\frac{K}{S} = \frac{(1 - R_d)^2}{2R_d} = a,
\]

(2)

with \(K\) being the absorption KM coefficient and \(S\) the scattering KM coefficient. Considering a thin slab of tissue of thickness \(t\) we get Equation (3).

\[
K = 2\sigma_a,
\]

\[
S = \frac{1}{bt} \ln \left( \frac{1 - R_d(a - b)}{T_d} \right),
\]

(3)

with \(b = \sqrt{a^2 - 1}\). Using Equation (3) and Equation (2) the absorption coefficient \(\sigma_a\) could be estimated and combining the results with Equation (1) the experimental value for the scattering coefficient \(\sigma_s\) could also be obtained.

3. Results and discussion

Collimated transmittance measurements of the first experiment for different lipid concentrations are shown in Figure 4, these spectra show similar behavior for all three types of milk, being monotonously increasing with the wavelength of light. There are however, intensity and shape spectral differences from whole milk to lactose-free and to fat-free, and this last one exhibits the biggest light transmittance as expected. With the data obtained, the dependence between the total extinction coefficient and the wavelength could be plotted using the Beer-Lambert law for a sample of 1 cm thickness as presented in Figure 5.

The diffuse reflectance spectra shown in Figure 6 was measured for four lipid concentrations and exhibit a monotonous decreasing behavior, but also in this case there are noticeable differences between the types of milk and variations with the composition of the model. In the diffuse transmittance measurements in Figure 7 spectral shape variations are small, although there are value differences for the three milk kinds and for the milk concentration of the phantoms.
Figure 4. Collimated transmittance vs light wavelength for phantoms with different concentrations. (a) Whole 3% fat milk. (b) Lactose-free 1.75% fat. (c) Fat-free 0% fat.

Figure 5. Total extinction coefficient vs wavelength for different lipid concentrations. (a) Whole 3% fat milk. (b) Lactose-free 1.75% fat. (c) Fat-free 0% fat.

Figure 6. Diffuse reflectance measurements for different phantom concentration values. (a) Whole 3% fat milk. (b) Lactose-free 1.75% fat. (c) Fat-free 0% fat.

With the diffuse measurements data obtained dependence between Kubelka Munk’s ratio K/S on the wavelength could also be plotted as presented in Figure 8. Optical properties of the phantoms could be determined using the method presented by Van Gemert, et al. [11], the experimental values for the absorption coefficient $\sigma_a$ and scattering coefficient $\sigma_s$ for phantoms with 1.0% lipid concentration were calculated and their dependence with the wavelength was plotted as shown in Figure 9 with $\sigma_s$ presenting higher values than $\sigma_a$ as expected since these liquid lipid based phantoms are turbid media [12].
Collimated transmittance measurements for the milk phantoms with the addition an absorbing material are shown in Figure 10. Absorption due to red India ink is presented around $555 \pm 0.3$ nm and the effect of each component is observed, i.e., in Figure 10(a) absorption increases as ink concentration is higher and in Figure 10(b) scattering increases as milk concentration increases [13], and comparing with the $T_c$ for lipid only the same increasing behavior with the wavelength is seen.
Figure 10. Collimated transmittance measurements. (a) 0.1% lipid increasing ink concentration, and (b) 0.2% ink increasing lipid concentration.

Dependence between the extinction coefficient and the wavelength for a 0.1% milk phantom with different ink concentrations could be plotted, Figure 11.

Figure 11. Total extinction coefficient vs wavelength for different ink concentrations.

Fluorescence was observed only for blue violet 405 nm and green 532 nm laser and LED sources and the spectra as shown in Figure 12. In the first image there are three samples: demineralised water only, water and ink and water, ink and milk and in the second image water, water+ink and water+milk. Light propagation processes such as transmittance, fluorescence, absorption and diffusion can be seen in this images and further analysis could be done. The fluorescence spectra measured is presented in Figure 13 presenting a peak at 586 nm with a 31 ± 0.3 nm Stroke’s shift.

Figure 12. Fluorescence observed.
4. Conclusions

In this study, low cost liquid lipid based phantoms with commercial milk and India ink were successfully made. From the collimated and diffuse spectroscopic measurements optical properties of tissue-like phantoms could be estimated, dependence between extinction coefficient and Kubelka Munk’s ratio on the wavelength was obtained. From these, the possibility of spectroscopic identification and control of milk kinds is demonstrated. Phantoms’ absorption and scattering coefficient could be estimated using a simple protocol based on a slab geometry for the diffusion approximation, assuming that the index of refraction of the phantom is equal to that of its environment. Absorption and fluorescence effects due to the red India ink were observed. This work illustrates the feasibility of a low cost controllable phantom which allows its use to learn the techniques of diffuse spectroscopy and its application to optical characterization of turbid media. Altogether, demonstrates a low cost system for preliminary studies in Biomedical Optics and Biophotonics.

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