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Article *in* Laser Physics · February 2015

DOI: 10.1088/1054-660X/25/2/025605

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2015 Laser Phys. 25 025605

(http://iopscience.iop.org/1555-6611/25/2/025605)

IOP Publishing | Astro Ltd Laser Physics

Laser Phys. **25** (2015) 025605 (6pp) doi:10.1088/1054-660X/25/2/025605

Optical properties measurement of highly diffusive tissue phantoms for biomedical applications

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Received 1 September 2014
Accepted for publication 19 November 2014
Published 24 December 2014

Abstract

Extensive research in biomedical optics essentially requires the determination of optical properties of various biological tissues. Quantitative characterization of biological tissues in terms of optical properties is achieved with an integrating sphere. However, samples having significantly higher scattering and absorption coefficients such as malignant tissues potentially reduce the signal-to-noise ratio (SNR) and the accuracy of an integrating sphere. We describe the design, implementation and characterization of a modified sample holder (path length of up to 1 mm) for an integration sphere. Experiments conducted with various phantoms reveal significant improvement of the SNR for a wide range of optical properties. The alternative approach opens up potential applications in the measurement of optical properties of highly diffusive biological samples. For 20% intralipid $\mu_a = 0.112 \pm 0.046\,cm^{-1}$ and $\mu_s = 392.299 \pm 10.090\,cm^{-1}$ at 632.8 nm. For 1.0% Indian ink $\mu_a = 9.808 \pm 0.490\,cm^{-1}$ and $\mu_s = 1.258 \pm 0.063\,cm^{-1}$ at the same wavelength. The system shows good repeatability and reproducibility within a 4.9% error.

Keywords: optical properties, integrating sphere system, body phantom, scattering and absorption coefficients

(Some figures may appear in colour only in the online journal)

1. Introduction

The study of light tissue interaction is gathering increased interest due to the growing number of biomedical applications such as fluorescence spectroscopy, optical coherence tomography (OCT) [1, 2], optical mammography, Raman spectroscopy [3], polarization imaging, photodynamic therapy (PDT) [4, 5], low-level laser therapy (LLLT) [6] etc. Tissue optical properties (absorption coefficients, scattering coefficients and anisotropy factor g) define light absorption and distribution in the sample and therefore have an important role in the study of light tissue interaction.

Tissue phantoms that mimic, simulate and easily tune optical properties of biological samples for various biomedical applications are highly desirable in the study of light tissue interaction. One of the most common phantoms used in biomedical applications is an aqueous solution of Intralipid (scattering medium) and Indian ink (absorbing medium). Proper dilutions of Intralipid and Indian ink reproduce phantoms with optical properties that mimic biological tissues, in terms of the absorption coefficient, scattering coefficient and anisotropy factor.

Optical properties are tuned and measured using integrating spheres (single or double) by the majority of researchers. Diffuse reflectance, diffuse transmittance and collimated transmittance are usually measured with integrating spheres. [7] Various algorithms like the Kubelka Munk model (KMM) [8], the inverse adding-doubling method (IAD) [9] and the

inverse Monte Carlo simulation (IMS) [10] are then applied for the extraction of the optical properties.

In spite of the many methodologies and the instrumentation proposed, the accurate measurement of optical properties using an integrating sphere with a standard sample chamber (1 cm path length) for high scattering and absorption coefficients of tissue phantoms remains a difficult task [11–13]. The signal-to-noise ratio (SNR) for phantoms simulating such highly diffusive cases is significantly smaller for the integrating sphere. For instance, malignant tissues with nuclear enlargement, increased chromatin content and high cellular density have a higher refractive index and scattering and absorption coefficients. An increase in absorption coefficients of 56.8% for adenomatous human colon mucosa/sub mucosa compared to normal has been reported. The higher scattering coefficient of an infiltrative basal cell carcinoma compared to a normal cell is explained by the structural characteristics. Biological tissues and/or phantoms of 1 cm path length that mimic malignant tissue would be highly diffusive resulting in a potentially smaller SNR [14, 15]. Consequently, the determined optical properties may not have the desired accuracy.

Optical properties of tissue 'thin' slabs and phantoms simulating such tissues are important for medical applications in diagnosis and therapy. In this study, we present the design, implementation and characterization of a modified sample holder (path length of 1 mm) for an integrating sphere that could be conveniently used for thin tissue samples and phantoms of high scattering and absorption coefficients. The reduced light path length significantly improves the signal-to-noise ratio which is validated for various tissue phantoms. Optical properties were measured at 632.8 nm using the inverse adding-doubling (IAD) algorithm [9, 16–18]

2. Material and method

Many biomedical applications of lasers depend on optical properties of biological tissue. The distribution of light within tissues is strongly influenced by the scattering coefficient while the conversion of photon energy to thermal energy depends on the absorption coefficient of tissue. Therefore, accurate knowledge of tissue optical properties is necessary for the study of light tissue interaction. In this work, we have implemented the IAD algorithm to extract the absorption coefficient (μ_a) and scattering coefficient (μ_s) from the measured value of diffuse reflectance R_d and diffuse transmittance T_d of tissue phantoms made from various dilutions of Intralipid and Indian ink.

2.1. Sample chamber

A specially designed cylindrical shape sample chamber was fabricated from aluminum with a central hole of 10 mm diameter for the integrating spheres as shown in figure 1(*a*)–(*f*). The chamber has a slot of thickness 1.4 mm at right angles to the light beam for sample placement. A phantom solution was put between two back-to-back stacked micro-glass slides. Each micro-slide is 135 μm thick (Deckglaser Germany) and separated by 1 mm (path length of 1 mm). Various tissue phantoms with different concentrations of Intralipid and Indian ink were placed between the integrating spheres to measure the diffuse transmittance and diffuse reflectance. It is worth mentioning that the separate micro-glass slide holder was fabricated and used for each phantom measurement.

2.2. Phantom preparation

Intralipid (20%) was purchased from Braun AG 34209 Melsungen, Germany and diluted with distilled water to prepare an Intralipid 10% stock solution. Serial dilutions were used to prepare ten different concentrations of Intralipid (0.001 to 0.01%). Similarly, a stock solution of 1.0% Indian ink (Perkin) was diluted with distilled water to prepare ten different concentrations (0.01 to 0.1%). In the next step, ten different Intralipid phantoms (concentration varied from 0.5 to 6.8%) were prepared with a fixed Indian ink concentration so that the scattering coefficient changed while the absorption coefficient remained constant. Finally, various Indian ink phantoms (5–50μl/5 ml) were prepared with a fixed or optimized Intralipid concentration.

2.3. Refractive index, diffuse reflectance and diffuse transmittance measurement

The refractive indices of the Intralipid and Indian ink dilutions were determined at 632.8 nm by angle of minimum deviation δm techniques using an equilateral hollow prism made of quartz slides 1 × 1 cm^2 (HELMA, Germany) [8,19] as shown in figure 2. Details of the experimental set-up to measure δm can be found elsewhere [15]. The refractive index was calculated from the measured δm using the following relations:

$$\delta \mathbf{m} = \tan^{-1}\left(\frac{Y}{X}\right) \tag{1}$$

$$\mathbf{n} = \frac{\sin\left(\frac{\mathbf{A}+\delta \mathbf{m}}{2}\right)}{\sin\left(\frac{\mathbf{A}}{2}\right)} \tag{2}$$

where A (= 60°) is the prism angle, δ_m is the angle of minimum deviation and Y measures the horizontal distance on the screen from the central to the deviated beam position after passing through the given phantom solution. The determined value of the refractive index for 20% Intralipid and 1.0% Indian ink was 1.481 and 1.356, respectively.

Diffuse reflectance R_d and diffuse transmittance T_d were measured using two identical integrating spheres (Optoprim, Germany) of 300 mm diameter. The specially designed sample chamber was sandwiched between the flanges connecting the two integrating spheres. A He–Ne laser (Melles and Griot LHR_25, 632.8 nm, 0.96 mm beam diameter at FWHM, 0.84 mRad beam divergence and 17 mW output powers) was used as the light source. A sketch of the experimental set-up is presented in figure 3.

The laser beam irradiates the phantoms after passing through the entrance port (10 mm diameter) of integrating

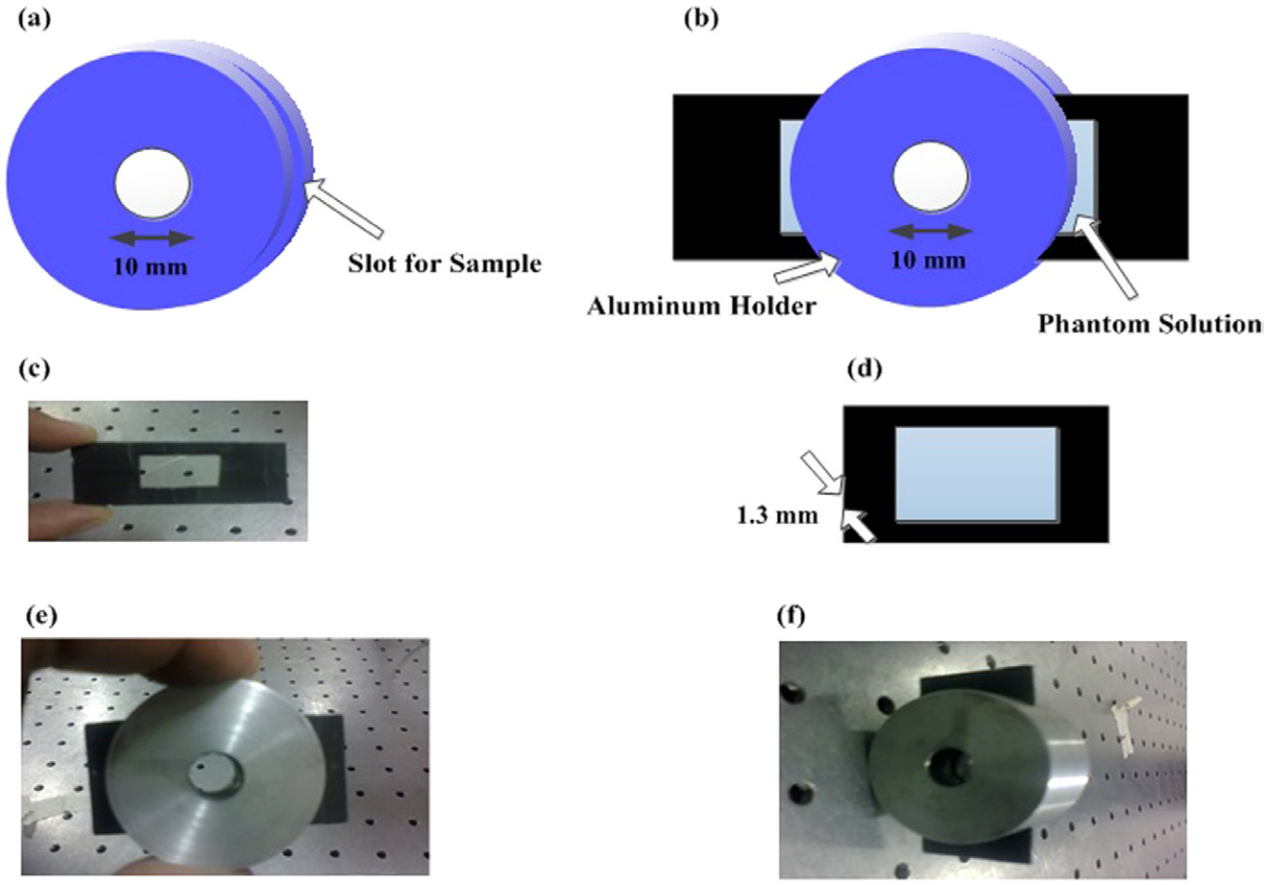

Figure 1. Sketch of a modified sample chamber for an integrating sphere inserted into an aluminum holder without (*a*) and with (*b*) a phantom solution. Empty sample chamber (*c*), filled sample chamber with phantom material (*d*). Front (*e*) and side (*f*) view of the aluminum sample holder with the phantom solution in position.

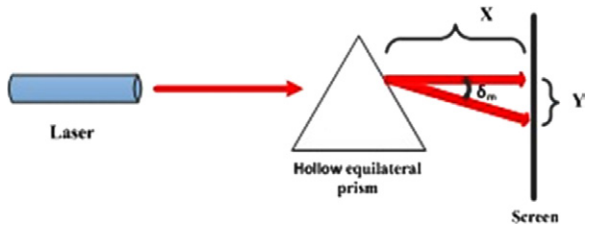

Figure 2. Set-up to measure the refractive index of Intralipid and Indian ink.

sphere 1. Diffuse reflectance R_d and diffuse transmittance T_d signals were collected at measuring port 1 and 2 respectively of the integrating spheres by Avaspec photo spectrometer (Avaspec, Russia, 2048 pixels, 600 lines mm^{-1}, 2 nm resolution CCD linear array). Reflecting baffles within the spheres secured the spectrophotometer from direct light exposure. Diffuse reflectance R_d and diffuse transmittance T_d were calculated using the equations [8]

$$R_d = \frac{X_r - Y_r}{Z_r - Y_r} \tag{3}$$

$$T_d = \frac{X_t - Y_t}{Z_t - Y_t} \tag{4}$$

where R_d is diffuse reflectance, T_d is diffuse transmittance, X_r and Z_r are measured light intensities with sample and standard reflector (barium sulfate) at port 1, respectively. Y_r is the stray light detected without any sample at port 1. Similarly X_t and Z_t are light intensities measured at port 2. Y_t is the stray light detected at port 2 without any sample.

3. Results and discussion

Intralipid composed of soybean oil encapsulated within a monolayer membrane of lecithin (hence the name Intralipid) is assumed as a pure scattering medium. Indian ink is considered as a pure absorber although a small scattering coefficient has been reported. A combination of proper dilutions of Intralipid and Indian ink can therefore produce a phantom with desirable optical properties as described in many studies [4, 20–24].

To calibrate the system for the specially designed sample holder, various dilutions of Intralipid and Indian ink were used. An attempt was made to validate the system in the typical range of optical properties of most biological tissues. A linear increase in the scattering coefficient (from 5 to 85 cm^{-1}) was observed as the concentration of Intralipid was increased from 0.5% to 6.8%. It may be noted that the scattering coefficient of most biological tissue lies within this range [4, 25] as shown in figure 4(*a*)–(*e*). The absorption coefficient of Intralipid showed no dependence on the concentration as reported by many other studies. For Indian ink, an almost linear increase

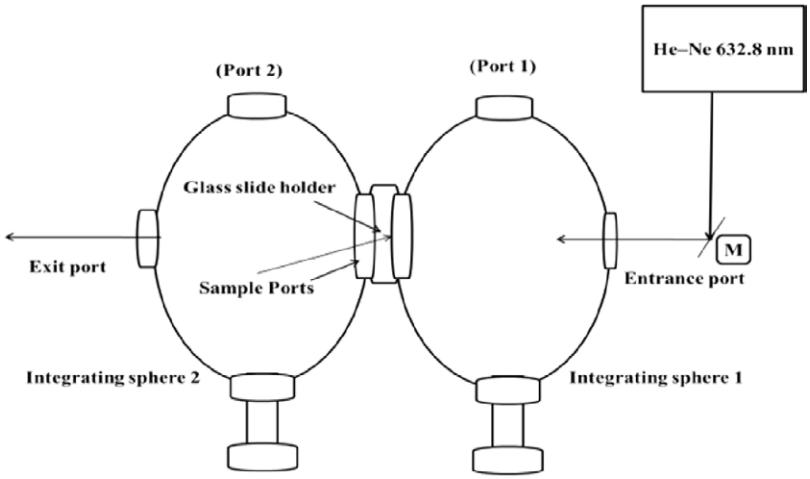

Figure 3. Experimental set-up for the whole experiment to measure R_d and T_d.

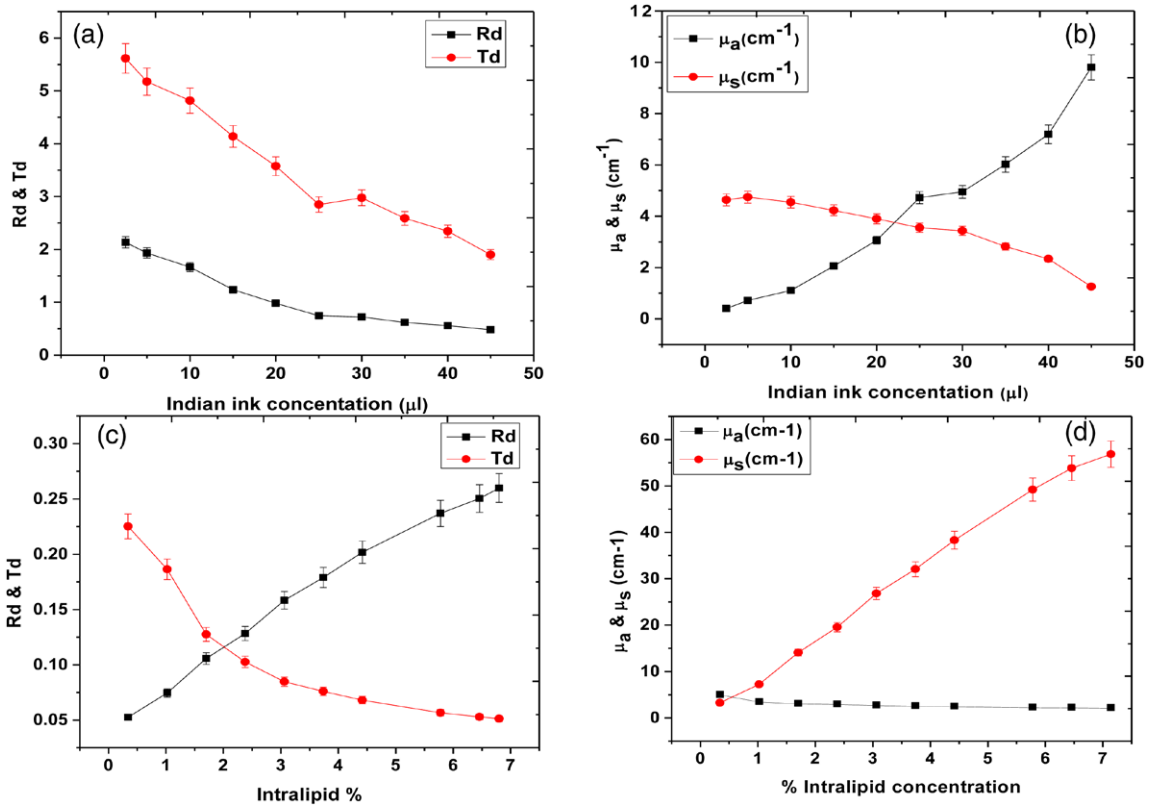

Figure 4. Diffuse reflectance (Rd) and transmittance (Td) for various Intralipid (*a*) and Indian ink (*c*) concentrations. The corresponding absorption (μ_a) and scattering coefficients (μ_s) of Intralipid (*b*) and Indian ink (*d*) measured at $\lambda = 632.8$ nm.

was observed for the absorption coefficient while the scattering coefficient decreased as reported recently.

Total attenuation coefficient μ_t was calculated from the Gaussian profile of the beam intensities using the Lambert beer law figure 5. For Intralipid, the total attenuation coefficient increased from 0.2 to 1.2 cm^{-1} as the concentration was varied from 0.001 to 0.01%. For Indian ink, the total attenuation coefficient increased faster (from 0.2 to 4.0 cm^{-1}) with concentration (0.01% to 0.1%).

The total attenuation coefficient increased linearly with concentrations for both cases. However, the slope was higher for Indian ink as compared to Intralipid, reflecting the difference in constituents of both samples. Even a small change in bubbles can effect the scattering coefficient as reported by [26]

The system with a modified sample chamber and holder has been validated at the highest available concentration of Intralipid 20%. Results are presented in table 1 and table2. The measured scattering coefficient of Intralipid 20% with our system is 392.299 ± 10.090 at 632.8 nm. It is interesting to note that the absorption coefficient of Intralipid 20% is negligibly small. The results are in good agreement with the reported values of many studies [27].

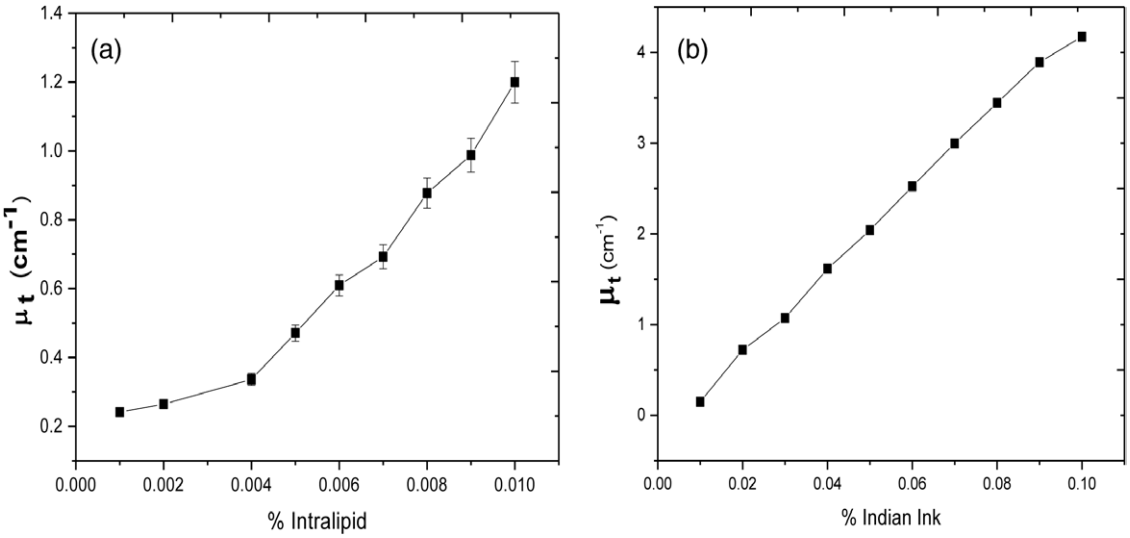

Figure 5. Linearity of the total attenuation coefficient μt (cm^{-1}) (*a*) Intralipid (0.001 to 0.01%) (*b*) Indian ink (0.01 to 0.1) at $\lambda = 632.8$ nm.

Table 1. Optical properties (R_d, T_d, n) of Intralipid (20%) and Indian ink (1.0%) at 632.8 nm.

Phantom	R_d	T_d	n
Intralipid 20%	0.869 ± 0.004	0.117 ± 0.003	1.481
Indian ink 1.0%	0.478 ± 0.024	1.90122 ± 0.095	1.356

Table 2. Optical properties (μ_a, μ_s, g) of Intralipid (20%) and Indian ink (1.0%) at 632.8 nm.

Phantom	μ_a (cm^{-1})	μ_s (cm^{-1})	g
Intralipid 20%	0.112 ± 0.046	392.299 ± 10.090	0.59
Indian ink 1.0%	9.808 ± 0.490	1.258 ± 0.063	0.90

The measurement of optical properties for a highly diffusive phantom such as Intralipid 20% with a standard sample chamber (1 cm path length) generates an unacceptably small signal-to-noise ratio. Tissues with high scattering and absorption coefficients would face similar problems. It has been reported that absorption and scattering coefficients increase many fold for cancerous tissues. Consequently, the integrating sphere system imposes limitations on the measurement of optical properties in many important scenarios. Our modified sample holder has a maximum path length of 1 mm and can be used conveniently for optical properties of such highly diffuse tissues/phantoms. The system with a modified sample holder shows excellent repeatability and reproducibility.

4. Conclusion

The design, calibration and validation of a modified sample holder (path length of 1 mm) for an integrating sphere system is presented. High signal-to-noise ratio for thin tissue samples and phantoms of large scattering and absorption coefficients could be obtained conveniently with the modified sample holder. The system is validated for various tissue phantoms of Intralipid and Indian ink and is found to be in good agreement with other studies. The modified system would have important biomedical applications in both photodiagnosis and photodynamic therapy of biological tissues.

Acknowledgement

We extend our special gratitude to Mr Saeed ul Hassan, Principal Technician (NILOP) for his technical assistance.

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