Effect of dependent scattering on the optical properties of Intralipid tissue phantoms

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Abstract: The calibration of optical tissue-simulating phantoms remains an open question in spite of the many techniques proposed for accurate measurements of optical properties. As a consequence, a reference phantom with well known optical properties is still missing. As a first step towards a reference phantom we have recently proposed to use dilutions of Intralipid 20%. In this paper we discuss a matter that is commonly ignored when dilutions are prepared, i.e., the possibility of deviations from the simple linear relationships between the optical properties of the dilution and the Intralipid concentration due to the effects of dependent scattering. The results of an experimental investigation showed that dependent scattering does not affect absorption. As for the reduced scattering coefficient the effect can be described adding a term proportional to the square of the concentration. However, for concentrations of interest for tissue optics deviations from linearity remain within about 2%. The experimental investigation also showed that the microphysical properties of Intralipid are not affected by dilution. These results show the possibility to easily obtain a liquid diffusive phantom whose optical properties are known with error smaller than about 1%. Due to the intrinsic limitations of the different techniques proposed for measuring the optical properties it seems difficult to obtain a similar accuracy for solid phantoms.

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1. Introduction

Many different methods have been presented over the past two decades for measuring the optical properties of diffusive media based on both measurements in the time domain [1–3], in the frequency domain [4, 5], and in the CW domain [6–9]. However, as also shown by recently published results [10, 11], in spite of the many methodologies and instrumentation proposed,

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the accurate determination of the optical properties of diffusive media remains a difficult task. Ref. [10] reports the results of a multi-laboratory experiment: The application of the MEDPHOT protocol in measuring the optical properties of the same phantom with eight different instrumentation revealed differences of about 40% for the reduced scattering coefficient (μ_s') and 30% for the absorption coefficient (μ_a). In Ref. [11], devoted to the development of a solid reference phantom for diffuse optical spectroscopy, refined measurements of time resolved transmittance have been inverted using a lookup table based on Monte Carlo results and the different sources of error have been carefully investigated. From the results presented it seems difficult to understand the ultimate reason for the relatively large error (about 6% for the reduced scattering coefficient and 11% for the absorption coefficient) that remains on the results.

Difficulties in obtaining accurate measurements of optical properties of diffusive media are intrinsic to the relationships between μ_a and μ_s' and the measured quantities. Usually μ_a and μ_s' are obtained from measurements of diffuse reflectance or of diffuse transmittance, and for the difficulties in calibrating the instrumentation measurements are usually available only in arbitrary units. Without the information on the absolute values for both time domain, frequency domain, and multidistance CW measurements the strong correlation between μ_a and μ_s' makes the non-linear inversion procedure very sensitive to experimental errors. The intrinsic difficulties related to the inversion procedures can be overcome if relative measurements of diffused light can be repeated after changing in a well controlled way the absorption or the reduced scattering coefficient of the medium, adding for instance a calibrated absorber, or changing the concentration of scatterers. In this case, between the optical properties to be determined and the relative variations of the measured quantities a linear relationship can often be obtained with a low correlation between μ_a and μ_s' leading to robust and accurate results [12, 13].

As a consequence of the difficulties in measuring the optical properties a reference phantom with well known optical properties is still missing, in spite of the many different materials proposed especially for tissue-simulating phantoms [14]. We point out that reference phantoms are necessary tools for studying photon migration, for the development, validation, and calibration of biomedical optical instrumentation [14] and, more in general, of devices for quality control of agricultural [15], pharmaceutical products [16], etc. In a recently published paper [17] we proposed the use of Intralipid 20% as a first step towards a reference diffusive medium for tissue-simulating phantoms. The proposal originated from measurements of optical properties on many samples from nine different batches produced over a period of ten years that showed a high temporal stability and surprisingly small batch-to-batch variations: At NIR wavelengths, for the reduced scattering coefficient, deviations from the value averaged over the nine batches were within 1.5%, and the results for the absorption coefficient were slightly smaller with respect to absorption of pure water [17]. It might be therefore useful to measure as precisely as possible the optical properties of Intralipid. In Ref. [12] a method has been presented based on measurements of effective attenuation coefficient (μ_{eff}) for different concentrations of Intralipid in water (method of water absorption). Making the assumption that the optical properties of the dilution are related to the concentration by linear relationships, Intralipid can be calibrated exploiting the knowledge of the absorption coefficient of water. The accuracy of the method is ultimately limited by the error on the absorption coefficient of water and by the assumption of the linear relationships between the optical properties and the concentration.

The possibility of deviations from the linear relationships is commonly ignored in evaluating the optical properties of dilutions, but it should be reminded that the linear relationships are expected to be applicable only if 1) the microphysical properties of suspended particles (size distribution, shape, and refractive index) are not affected by dilution in water, and 2) the independent scattering approximation is fulfilled [18, 19]. The first assumption ensures that the

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optical properties of suspended single particles (scattering function, scattering and absorption cross section) are independent of the concentration of diffusive medium. The second assumption ensures that suspended particles act as independent and uncorrelated scatterers, so that the power scattered (absorbed) by the unit volume is the sum of the power scattered (absorbed) by each particle. If both the assumptions are fulfilled the scattering and absorption coefficients due to suspended particles are proportional to the number of particles in the unit volume and thus to the volume concentration of particles, whereas the scattering function is independent of the concentration.

In Ref. [12] the effect of dependent scattering on measurements carried out at λ = 751 nm has been discussed making use of experimental results obtained at concentrations significantly higher than concentrations of interest for tissue simulating phantoms, and at a shorter wavelength (λ = 632.8 nm) [20], whereas the possibility that the microphysical properties of scattering particles depend on the concentration has not been discussed at all. This paper is devoted to a deeper investigation of these effects. Experimental results showed that dilution of Intralipid in water does not appreciably change the microphysical properties of suspended particles, and that dependent scattering does not affect the absorption coefficient. For the reduced scattering coefficient small deviations from linearity have been observed that can be accounted for with a term proportional to the square of the concentration. Anyway, for concentrations necessary to obtain the values of $\mu_s^{'}$ of practical interest for tissue phantoms ($\mu_s^{'} < 2$ mm^{-1}) deviations remain within about 2%.

The procedure used to investigate the effect of dependent scattering needs to use an absorber: We used India ink, after having checked that it can be added without affecting the microphysical properties of Intralipid.

The methodology we used for the experimental investigation is described in section 2; the experimental results are presented in section 3 and conclusions are in section 4.

2. Materials and Methods

2.1. Monitoring the Microphysical Properties of Diluted Intralipid

Intralipid consists of fat droplets suspended in water that make it a highly scattering medium [21]. To investigate if the microphysical properties of fat droplets change when Intralipid 20% is diluted in water or when an absorber is added, we carried out measurements of extinction coefficient. With reference to spherical droplets, and making the assumption of independent scattering [18], the extinction coefficient μ_e due to Intralipid is related to the scattering and absorption properties of suspended droplets by [22]

$$\mu_e(\rho_{il}) = N \int_0^\infty C_{eil}(\frac{r}{\lambda}, n) f(r) dr \tag{1}$$

where $f(r)$ is the particle size distribution, $N f(r)dr$ is the number of droplets in the unit volume with radius between r and $r + dr$, and C_{eil} is the extinction cross section. C_{eil} depends on the relative refractive index n of fat droplets with respect to water, and on the ratio between the size of the droplet and the wavelength. Since N is proportional to the volume concentration ρ_{il} of Intralipid 20% diluted in water, μ_{eil} can be written as

$$\mu_e(\rho_{il}) = \varepsilon_{eil} \rho_{il} \tag{2}$$

where the coefficient ε_{eil} is the specific extinction coefficient of Intralipid 20%, determined by the concentration of fat droplets, by their size distribution, and their refractive index.

Measurements of ε_{eil} can therefore be used to monitor any change in the microphysical properties of fat droplets due to the dilution of Intralipid 20% in water or to the addition of

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absorbers: If dilution or addition of absorbers affects the size distribution or the refractive index of fat droplets we expect to obtain different values of ε_{eil} from measurements carried out on dilutions prepared in different ways.

The specific extinction coefficient has been obtained from measurements of collimated transmittance on samples with moderate turbidity ($\mu_e < 0.05$ mm^{-1}). Intralipid was diluted inside a scattering cell and the transmitted power $P(\rho_{il})$ has been measured for different concentrations. Inverting the Lambert-Beer law the value of the extinction coefficient has been obtained for each concentration as

$$\mu_e(\rho_{il}) = \frac{1}{L} \ln \frac{P(\rho_{il} = 0)}{P(\rho_{il})}, \tag{3}$$

where L is the thickness of the cell, and ε_{eil} has been determined from the slope of the straight line that best fits $\mu_e(\rho_{il})$ as a function of ρ_{il}.

2.2. Dependent Scattering

The independent scattering approximation is applicable provided the average distance between scatterers is sufficiently large with respect to the wavelength [18]. Experimental investigations [19, 20] showed that for particles with radius smaller than the wavelength the effect of dependent scattering becomes significant when the volume concentration of scatterers is larger than 0.01. Since the volume concentration of fat droplets of Intralipid 20% necessary to obtain the values of $\mu_s^{'}$ of interest for tissue phantoms can be larger than 0.01 (as an example, the volume concentration of Intralipid 20% necessary to obtain $\mu_s^{'}$ = 2 mm^{-1} at λ = 751 nm is $\rho_{il} \cong 0.1$, that corresponds to a volume concentration of fat droplets of $\cong$ 0.022), to obtain phantoms with well calibrated optical properties it is therefore necessary to check if the dependent scattering leads to significant deviations from the linearity.

The procedure we used to investigate the effect of dependent scattering at NIR wavelengths is based on measurements of effective attenuation coefficient $\mu_{eff} = \sqrt{3\mu_a\mu_s^{'}}$ of dilutions with high turbidity of Intralipid and India ink in water. μ_{eff} has been obtained from multidistance measurements of fluence rate $\phi(r)$ carried out in an infinite medium illuminated by an isotropic CW source [20]. From the solution of the diffusion equation for the fluence at distance r from a point source emitting a unitary power:

$$\phi(r) = \frac{3\mu_s^{'}}{4\pi r} \exp(-\mu_{eff} r), \tag{4}$$

we obtain

$$\ln[r\phi(r)] = \ln \frac{3\mu_s^{'}}{4\pi} - \mu_{eff} r, \tag{5}$$

and μ_{eff} is obtained from the slope of the straight line that best fits $\ln[r\phi(r)]$ as a function of the source-receiver distance r. The overall procedure can be summarized in 6 steps.

Step 1: The optical properties of Intralipid 20% are first calibrated using the method of water absorption [12], which consists in measuring μ_{eff} as a function of the concentration of Intralipid diluted in water. With the assumption that the independent scattering approximation is applicable and that dilution does not affect the microphysical properties of fat droplets, the reduced scattering and the absorption coefficients of the dilution are related to ρ_{il} by

$$\mu_s^{'}(\rho_{il}) = \varepsilon_{sil}^{'} \rho_{il} \tag{6}$$

$$\mu_a(\rho_{il}) = \varepsilon_{ail}\rho_{il} + \varepsilon_{aH_2O}(1-\rho_{il}) \tag{7}$$

and

$$\mu_{eff}^2(\rho_{il}) = 3\varepsilon_{sil}^{'}\varepsilon_{aH_2O}\rho_{il} + 3\varepsilon_{sil}^{'}(\varepsilon_{ail} - \varepsilon_{aH_2O})\rho_{il}^2 \tag{8}$$

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where $\varepsilon_{sil}^{'}$ and ε_{ail} are the specific reduced scattering coefficient and the absorption coefficient of Intralipid 20% and ε_{aH_2O} is the absorption coefficient of pure water. Exploiting the knowledge of the absorption coefficient of water, $\varepsilon_{sil}^{'}$ and ε_{ail} are obtained as

$$\varepsilon_{sil}^{'} = \frac{I_{il}}{3\varepsilon_{aH_2O}} \tag{9}$$

$$\varepsilon_{ail} = \frac{S_{il}}{3\varepsilon_{sil}^{'}} + \varepsilon_{aH_2O} = \varepsilon_{aH_2O}(1+\frac{S_{il}}{I_{il}}) \tag{10}$$

where I_{il} and S_{il} are respectively the intercept and the slope of the straight line that best fits $\mu_{eff}^2(\rho_{il})/\rho_{il}$ as a function of ρ_{il}. These relations show that to get accurate measurements of $\varepsilon_{sil}^{'}$ and ε_{ail} the value of ε_{aH_2O} must be known with high accuracy.

Step 2: A sample of the Intralipid calibrated at step 1 is used to calibrate the absorption coefficient of India ink. The absorption coefficient is obtained from measurements of effective attenuation coefficient on a dilution of the calibrated Intralipid, as a function of the ink concentration ρ_{ink}. Denoting with $\mu_{sil}^{'}(\rho_{il})$ and $\mu_{ail}(\rho_{il})$ the reduced scattering and the absorption coefficient of the diluted Intralipid and with ε_{aink} the absorption coefficient of non diluted ink, μ_{eff}^2 can be written as

$$\mu_{eff}^2(\rho_{il},\rho_{ink}) = 3\mu_s^{'}(\rho_{il})(\mu_a(\rho_{il})+\varepsilon_{aink}\rho_{ink}) \tag{11}$$

where we assumed that the added ink does not appreciably change the Intralipid concentration and we neglected the contribution due to the scattering of ink. ε_{aink} is obtained as

$$\varepsilon_{aink} = \frac{S_{ink}}{3\mu_s^{'}(\rho_{il})} \tag{12}$$

where S_{ink} is the slope of the straight line that best fits $\mu_{eff}^2(\rho_{il},\rho_{ink})$ as a function of ρ_{ink}. We point out that if the effect of dependent scattering is significant Eq. (8) is approximated and the error on $\mu_s^{'}(\rho_{il})$ affects the value of ε_{aink}.

Step 3: Making use of the India ink calibrated at step 2, the method of adding absorption [20] is repeatedly applied to measure the optical properties of dilutions with different concentrations of Intralipid. For a given concentration ρ_{il} the method consists in measuring μ_{eff} after the addition of small quantities of the calibrated ink that changes the absorption coefficient of the dilution of known quantities $\Delta\mu_a(\rho_{ink}) = \varepsilon_{aink}\rho_{ink}$. Using Eq. (11), $\mu_s^{'}(\rho_{il})$ and $\mu_a(\rho_{il})$ are obtained as

$$\mu_s^{'}(\rho_{il}) = \frac{S_{1ink}}{3\varepsilon_{aink}} \tag{13}$$

$$\mu_a(\rho_{il}) = \varepsilon_{aink}\frac{I_{1ink}}{S_{1ink}} \tag{14}$$

where S_{1ink} and I_{1ink} are respectively the slope and the intercept of the straight line that best fits $\mu_{eff}^2(\rho_{il},\rho_{ink})$ as a function of ρ_{ink}.

Step 4: To study deviations from linearity in Eqs. (6) and (7) due to dependent scattering, the values of $\mu_s^{'}(\rho_{il})$ and $\mu_a(\rho_{il})$ measured at step 3 are plotted as a function of ρ_{il}. Since, as it will be shown in Sect. 3.1, the microphysical properties of fat droplets are not affected by the dilution of Intralipid in water and by the addition of India ink, deviations from linearity can be ascribed to dependent scattering. As it will be shown by experimental results of Sect. 3.2, for concentrations of Intralipid 20% of practical interest for tissue phantoms deviations from the linearity of $\mu_s^{'}$ as a function of ρ_{il} are well described by

$$\mu_s^{'}(\rho_{il}) = \varepsilon_{s1\,il}^{'}\rho_{il} + \varepsilon_{s2\,il}^{'}\rho_{il}^2, \tag{15}$$

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with $\varepsilon'_{s2\,il}$ significantly smaller than $\varepsilon'_{s1\,il}$, while for $\mu_a(\rho_{il})$ no appreciable deviation from the linear behavior (Eq. (7)) has been observed. The coefficients $\varepsilon'_{s1\,il}$ and $\varepsilon'_{s2\,il}$ are obtained again with a linear fit from the intercept I_{1il} and the slope S_{1il} of the straight line that best fits $\mu'_s(\rho_{il})/\rho_{il}$ as a function of ρ_{il}. In particular the ratio $\varepsilon'_{s2\,il}/\varepsilon'_{s1\,il}$ can be obtained as:

$$\frac{\varepsilon'_{s2\,il}}{\varepsilon'_{s1\,il}} = \frac{S_{1il}}{I_{1il}}. \tag{16}$$

We point out that the ratio $\varepsilon'_{s2\,il}/\varepsilon'_{s1\,il}$ is not affected by the error on $\varepsilon_{ainк}$. In fact, the error on $\varepsilon_{ainк}$ causes a systematic overestimation or underestimation of $\mu'_s(\rho_{il})$ by a constant factor that does not affect the ratio $\varepsilon'_{s2\,il}/\varepsilon'_{s1\,il}$.

From the intercept I_{2il} and the slope S_{2il} of the straight line that best fits $\mu_a(\rho_{il})$ as a function of ρ_{il}, according to Eq. (7) we obtain: $\varepsilon_{aH_2O} = I_{2il}$ and $\varepsilon_{ail} = S_{2il} + I_{2il}$. We notice that these values are affected by the error on the absorption coefficient of India ink calibrated at step 2.

Step 5: The method of water absorption (step 1) is applied again, but using for $\mu'_s(\rho_{il})$ the expression that includes the effect of dependent scattering, i.e., using Eq. (15) instead of Eq. (6). Equation (8) changes to:

$$\mu^2_{eff}(\rho_{il}) = 3\varepsilon'_{s1\,il}\varepsilon_{aH_2O}\rho_{il} + 3[\varepsilon'_{s1\,il}(\varepsilon_{ail} - \varepsilon_{aH_2O}) + \varepsilon'_{s2\,il}\varepsilon_{aH_2O}]\rho^2_{il} + 3\varepsilon'_{s2\,il}(\varepsilon_{ail} - \varepsilon_{aH_2O})\rho^3_{il}. \tag{17}$$

With the values obtained for $\varepsilon'_{s1\,il}$, $\varepsilon'_{s2\,il}$, and ε_{ail} (see Sect. 3.2) it is possible to show that the contribution of the term proportional to ρ^3_{il} remains smaller than about 1% for all values of ρ_{il} at which measurements with the method of absorption of water have been carried out. This term can be therefore neglected in Eq. (17) and a new linear relationship between $\mu^2_{eff}(\rho_{il})/\rho_{il}$ and ρ_{il} is obtained, but with different coefficients with respect to Eq. (8). Using this new relationship, from the slope and the intercept of the line that best fits $\mu^2_{eff}(\rho_{il})/\rho_{il}$ as a function of ρ_{il} we obtain

$$\varepsilon'_{s1\,il} = \frac{I_{il}}{3\varepsilon_{aH_2O}} \tag{18}$$

$$\varepsilon_{ail} = \varepsilon_{aH_2O}\left(1 + \frac{S_{il}}{I_{il}} - \frac{\varepsilon'_{s2\,il}}{\varepsilon'_{s1\,il}}\right). \tag{19}$$

Comparison of Eqs. (18) and (19) with Eqs. (9) and (10) shows that the value of $\varepsilon'_{s\,il}$ obtained using the method of water absorption with the assumption of independent scattering, actually represents the term $\varepsilon'_{s1\,il}$ of Eq. (18), and that the correct value of ε_{ail} can be obtained using for the ratio $\varepsilon'_{s2\,il}/\varepsilon'_{s1\,il}$ in Eq. (19) the value obtained at step 4.

In conclusion, to include the effect of dependent scattering three parameters are necessary: $\varepsilon'_{s1\,il}$, $\varepsilon'_{s2\,il}$, and ε_{ail}. $\varepsilon'_{s1\,il}$ is obtained from Eq. (18), ε_{ail} from Eq. (19) using for $\varepsilon'_{s2\,il}/\varepsilon'_{s1\,il}$ the value obtained with the method of adding absorption, and $\varepsilon'_{s2\,il}$ from the values of $\varepsilon'_{s1\,il}$ and of $\varepsilon'_{s2\,il}/\varepsilon'_{s1\,il}$. Of the results obtained with the method of adding absorption we therefore use only the ratio $\varepsilon'_{s2\,il}/\varepsilon'_{s1\,il}$ that, as previously pointed out, is independent of the error on the calibration of India ink.

Step 6: Finally, a more accurate calibration for the specific absorption coefficient of the India ink can be obtained using in Eq. (12) (Step 2) the value of $\mu'_{s\,il}(\rho_{il})$ obtained from Eq. (15) that includes the effect of dependent scattering.

2.3. Experimental Setup

Measurements have been carried out using two CW laser diodes at λ = 751 nm and 833 nm. The emitted power was 3 and 30 mW respectively. To measure the extinction coefficient we have

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carried out measurements of collimated transmittance at different concentrations. We have used a scattering cell 34.5 mm thick, and transmittance was measured with a photodiode and a lock-in amplifier. The experimental setup was similar to that of Ref. [20] with an acceptance angle of the detection system of 7 mrad. With this small acceptance angle the error on the extinction coefficient due to the unavoidable fraction of scattered received power was negligible, and the specific extinction coefficient ε_{eil} has been obtained with an error smaller than 0.5%.

The setup for multidistance measurements of fluence in the infinite medium was similar to that of Ref. [20]. Two thin fibers with a small diffusive tip (outer diameter 0.5 mm) having a substantially uniform radiation pattern (US Patent Number 6,071,302 'Phototherapeutic apparatus for wide-angle diffusion') were used to illuminate the medium and to measure the fluence. The interfibre distance was varied between 10 and 35 mm (1 mm step) with a computer controlled translation stage and received photons were measured with a photomultiplier and a lock-in amplifier. The small error on the measured fluence (random error of about 0.1%) enabled us to obtain the effective attenuation coefficient with high accuracy (the standard deviation due to random errors was of less than 0.05%). Measurements have been carried out inside a cylindrical vessel with volume of about 3L. For the interfibre distances and for the values of $\mu_s^{'}$ and μ_a at which measurements have been carried out this volume was sufficiently large to act as an infinite medium. The volume concentration ρ_{il} has been obtained from the weight of Intralipid and water using the value 0.988 for the relative density of Intralipid 20% with respect to water [21]. The absorber we have used was a sample of prediluted Higgins India ink. Prediluition was necessary since, due to its high value of absorption coefficient (hundreds of mm^{-1}), it would be difficult to weigh with good accuracy the very small quantities of ink necessary to obtain the concentrations of interest for our measurements. For more detailed information on the use of India ink we refer to Ref. [23]. The concentrations both for Intralipid and for ink have been determined with error smaller than 0.1%.

3. Experimental Results

All the measurements have been carried out using different bags of Intralipid 20% from the same batch. As also shown in Ref. [17] we did not observe any significant difference in the optical properties of different bags.

3.1. Monitoring the Microphysical Properties of Diluted Intralipid

We have measured the specific extinction coefficient at λ = 751 nm and 833 nm carrying out measurements on dilutions with similar concentrations but prepared in different ways. Examples of results are reported in Fig. 1. Panels a)-c) pertain to λ = 751 nm, panels d)-f) to 833 nm. The error bars are not shown since smaller than the marks. Panels a) and d) show the results for a dilution prepared taking a sample directly from the bag of Intralipid 20%. To obtain accurate values for the concentrations the sample has been prediluted (1.185 g of Intralipid in 98.348 g of water).

Panels b) and e) show the results of extinction measurements on a sample of the dilution used for measuring the optical properties of Intralipid 20% with the method of water absorption (step 1). We have used a sample of the dilution in the cylindrical vessel at the end of measurements at λ = 751 nm (267.0 g of Intralipid 20% in 2478 g of water).

Panels c) and f) show the results of measurements on a sample of the dilution of Intralipid and India ink taken from the cylindrical vessel at the end of measurements at λ = 751 nm for step 2 (80.11 g of Intralipid 20% and 2.49 g of prediluted ink in 2458 g of water). The contribution to the extinction coefficient due to the small quantity of prediluted ink was negligible (smaller than 0.1%). The value expected for ε_{eil} from these measurements is therefore the same obtained from measurements on other samples

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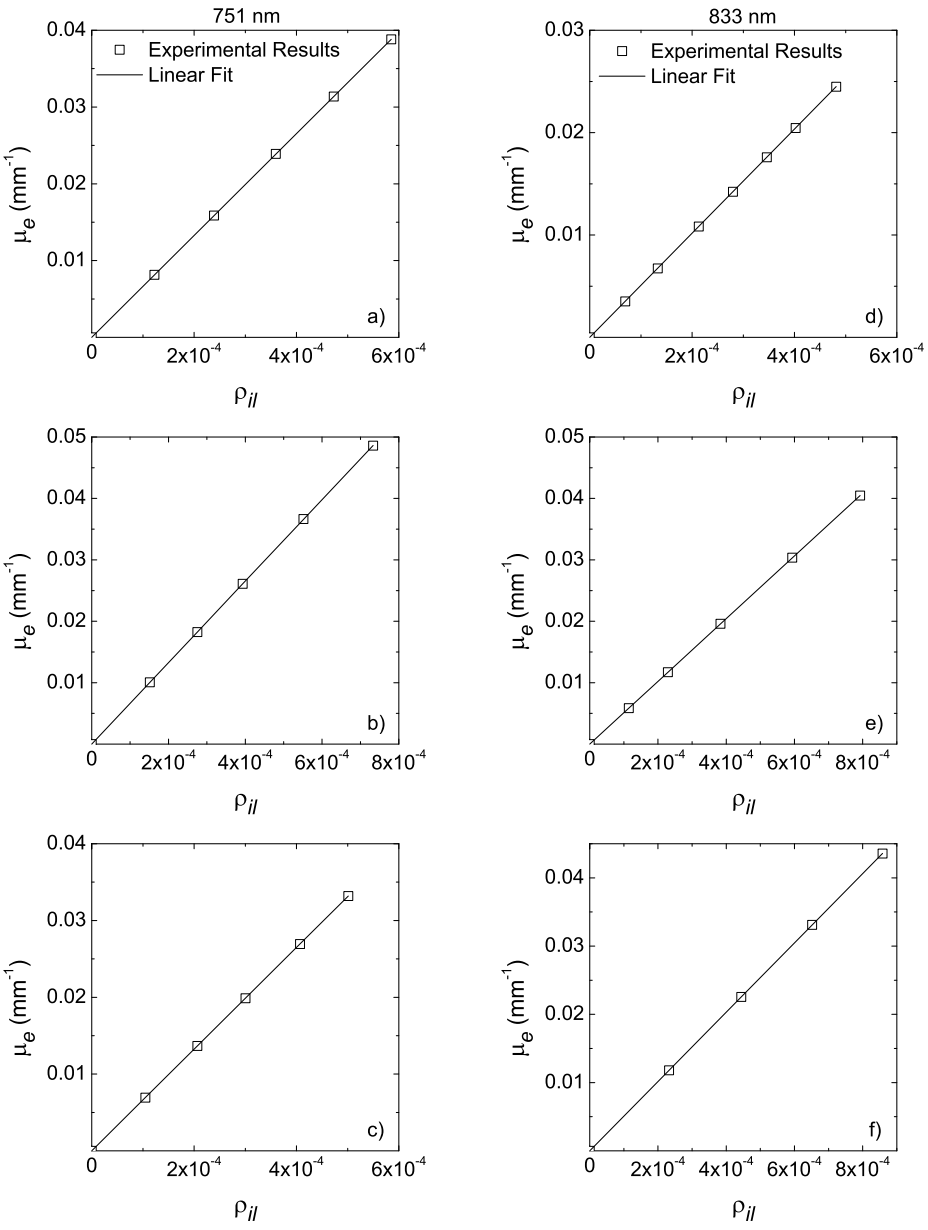

Fig. 1. Examples of measurements of collimated transmittance on dilutions of Intralipid 20% prepared in different ways. Panels a) and d): predilution with 1.185 g of Intralipid in 98.348 g of water; panels b) and e): predilution with 267.0 g of Intralipid in 2478 g of water; panels c) and f): predilution with 80.11 g of Intralipid and 2.49 g of prediluted ink in 2458 g of water. Panels a)-c) pertain to λ = 751 nm, panels d)-f) to 833 nm.

provided the added ink does not change the microphysical properties of Intralipid. The results we have obtained for ε_{eil} from measurements at 751 nm (panels a)-c)) were 66.3±0.3, 66.3±0.3, and 66.2±0.3 mm^{-1} respectively, and at 833 nm (panels d)-f)) 50.9±0.3, 51.1±0.3,

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and $50.8\pm0.3\,\mathrm{mm}^{-1}$ respectively. Within the standard deviation the results are indistinguishable both at 751 nm and at 833 nm, showing that the microphysical properties of diluted Intralipid do not depend on the way the dilution has been prepared. In particular, they are not affected by the addition of the India ink used for measurements at steps 2 and 3.

Measurements of collimated transmittance have been also carried out on dilutions of Intralipid 20% prepared using buffered water. To adjust the pH of water to the value (pH $\cong$ 8) measured for Intralipid [21] a small quantity of NaOH has been added. The results we have obtained were indistinguishable with respect to those obtained using purified water.

3.2. Dependent Scattering

The experimental results at λ = 751 nm and 833 nm are summarized in Figs. 2 and 3 respectively. Different panels show the results pertaining to the different steps of the measuring procedure together with the straight lines that best fit the results.

The results of panel a) are used to obtain $\varepsilon'_{s\,il}$ and $\varepsilon_{a\,il}$ with the method of water absorption (Eqs. (9) and (10), step 1). For the absorption coefficient of water, since published data show an appreciable spread of values and often the information on the standard error is not provided (see Ref. [12] for a deeper discussion), we have used the values obtained from measurements of attenuation in pure water. Using the method described in [12] we obtained ε_{aH_2O} = $(2.77\pm0.02)\times10^{-3}$ mm^{-1} and $(3.55\pm0.02)\times10^{-3}$ mm^{-1} at 751 and 833 nm respectively.

The results used to measure $\varepsilon_{a\,ink}$ (step 2) are plotted in panel b). The concentration of Intralipid 20% was ρ_{il} = 0.0289 at 751 nm and 0.0306 at 833 nm, and was not appreciably changed by the addition of the prediluted ink (ρ_{il} changed less than 0.1%).

Panels c) and d) show two examples of using the method of adding absorption to measure $\mu'_s(\rho_{il})$ and $\mu_a(\rho_{il})$ (step 3). Panel c) reports measurements for the smallest value of ρ_{il} we considered (ρ_{il} = 0.0204 at 751 nm and 0.0214 at 833 nm) and panel d) for the largest value (ρ_{il} = 0.1408 at 751 nm and 0.1082 at 833 nm). The values of $\mu'_s(\rho_{il})$ and $\mu_a(\rho_{il})$ measured at step 3 are displayed in panels e) and f) respectively. These data are used to evaluate the effect of dependent scattering (step 4). To highlight the small deviations from the linearity observed for μ'_s in panel e) we displayed the ratio $\mu'_s(\rho_{il})/\rho_{il}$. The ratio would be independent of ρ_{il} if the microphysical properties of Intralipid are not affected by the dilution or by the addition of India ink and the assumption of independent scattering (Eq. (6)) is fulfilled. Since it has been shown in Sect. 3.1 that dilution does not affect the microphysical properties, variations of the ratio $\mu'_s(\rho_{il})/\rho_{il}$ are therefore ascribed to the effect of dependent scattering. The experimental results show that $\mu'_s(\rho_{il})/\rho_{il}$ slightly decreases as ρ_{il} increases, i.e., the dependent scattering slightly decreases the reduced scattering efficiency. As anticipated in Sect. 2.2 the results are fitted reasonably well by a straight line, and the ratio S_{1il}/I_{1il} is used to obtain $\varepsilon'_{s2\,il}/\varepsilon'_{s1\,il}$ (Eq. (15)). Panel f) shows $\mu_a(\rho_{il})$ as a function of ρ_{il}. Also these results are fitted reasonably well by a straight line. This indicates that the dependent scattering does not appreciably affect absorption and also that absorption of Intralipid is smaller than absorption of water. From the slope and the intercept of the straight line the values of $\varepsilon_{a\,il}$ and ε_{aH_2O} are obtained using Eq. (7).

The results on the effect of dependent scattering are then used to reanalyze the data of panel a) and the final results for $\varepsilon'_{s1\,il}$, $\varepsilon'_{s2\,il}$, and $\varepsilon_{a\,il}$ are obtained (step 5). Finally, using for $\mu'_s(\rho_{il})$ the value evaluated with the results obtained at step 5), a more accurate value for $\varepsilon_{a\,ink}$ is obtained from the data of panel b) (step 6).

The results we have obtained are summarized in Table 1. The table shows the values obtained at different steps together with the corresponding standard deviation. The standard deviation has been evaluated considering only the contribution of random errors. We point out that Table 1 shows for $\varepsilon_{a\,il}$ both the results obtained at steps 4 and 5. The two values are consistent, however

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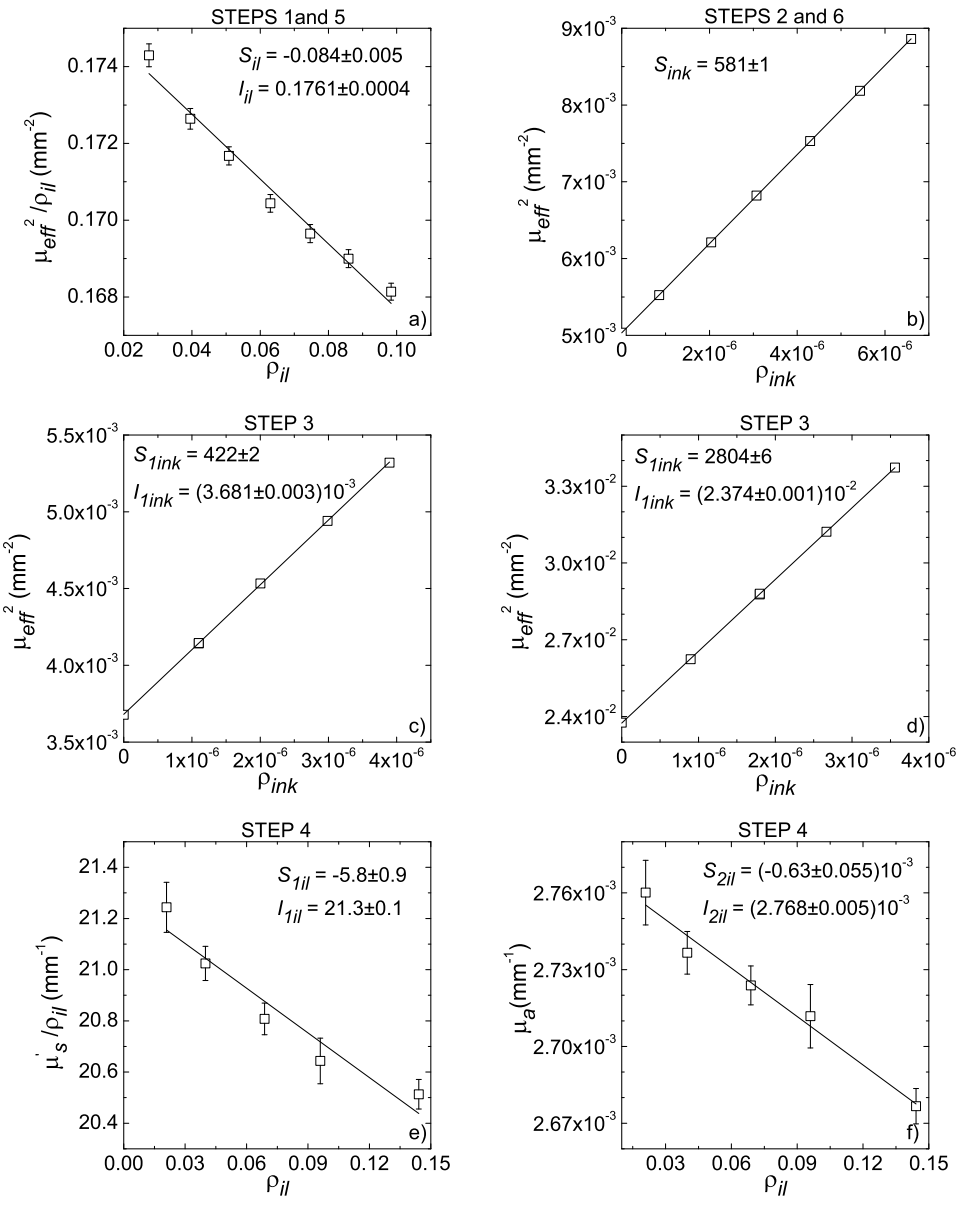

Fig. 2. Dependent scattering: Experimental results for λ = 751 nm. Each panel reports the experimental results (marks) together with the straight line that best fits the results. The error bars are shown only when larger than the marks. Panel a): results for μ_{eff}^2 as a function of ρ_{il} used to obtain ε_{sil}' and ε_{ail} with the method of absorption of water (step 1) and ε_{s1il}', ε_{s2il}', and ε_{ail} (step 5). Panel b) results for μ_{eff}^2 as a function of ρ_{ink} used to obtain ε_{aink} (steps 2 and 6). Panels c) and d): examples of results for μ_{eff}^2 as a function of ρ_{ink} used to obtain $\mu_s'(\rho_{il})$ and $\mu_a(\rho_{il})$ with the method of adding absorption (step 3) for ρ_{il} = 0.0204 and 0.1408 respectively. Panels e) and f): the results for $\mu_s'(\rho_{il})$ and $\mu_a(\rho_{il})$ of step 3 are plotted as a function of ρ_{il}. These results are used to evaluate the effect of dependent scattering (step 4)

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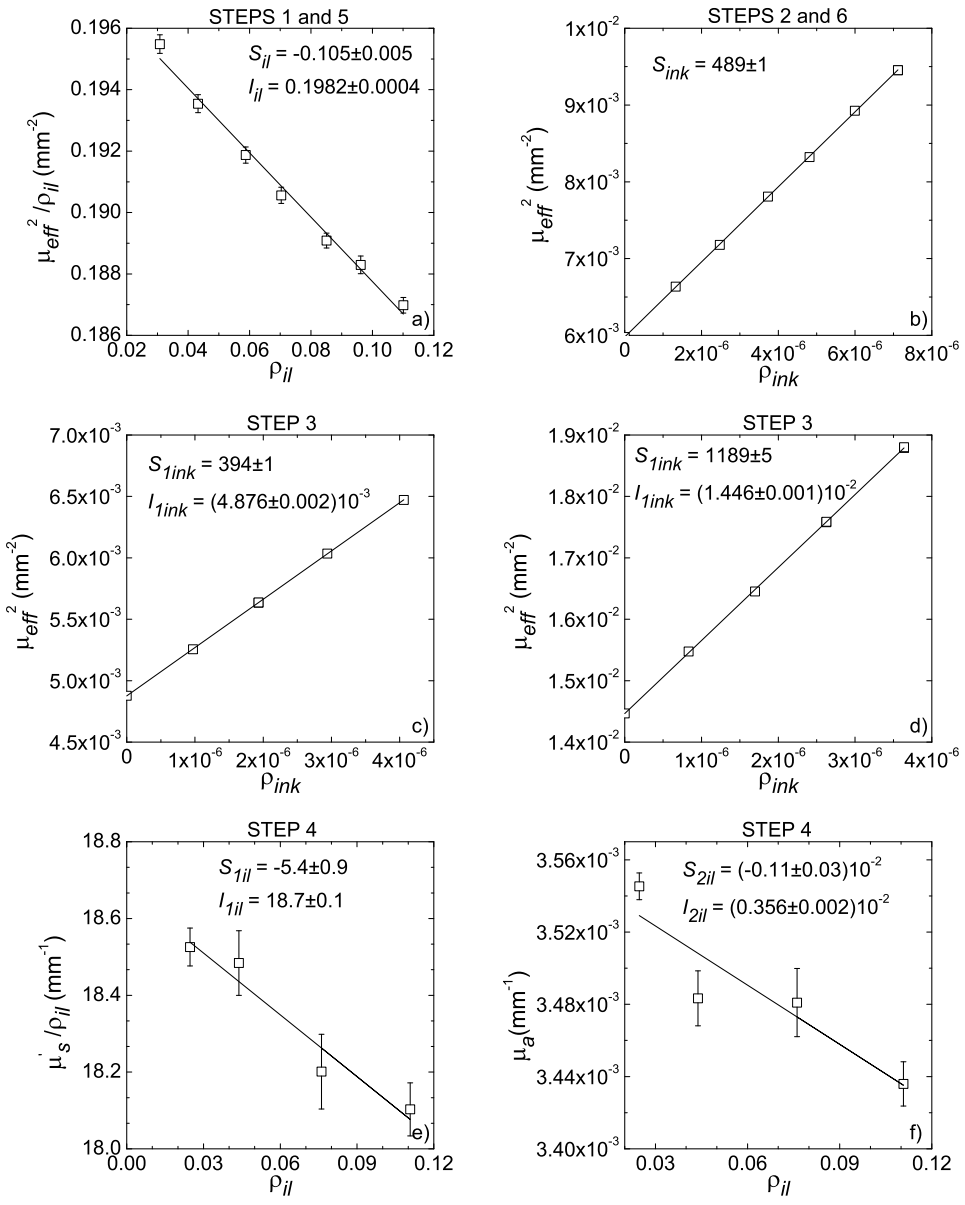

Fig. 3. Same as Fig. 2, but for λ = 833 nm. The results displayed in panels c) and d) refer to ρ_{il} = 0.0214 and 0.1082 respectively.

since the value obtained at step 4 is affected by the error on the calibration of India ink, the value obtained at step 5 is more reliable. We also note that the value of $\varepsilon_{a H_2O}$ obtained at step 4 is consistent with that obtained from direct attenuation measurements in pure water (section 3.2 and Ref. [12]).

#148955 - $15.00 USD Received 8 Jun 2011; revised 29 Jun 2011; accepted 6 Jul 2011; published 14 Jul 2011
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Table 1. Summary of the Experimental Results Obtained at Different Steps

	λ (nm)	751	833
STEP 1	$\varepsilon'_{sil}(\mathrm{mm}^{-1})$	21.16±0.04	18.57±0.03
	$\varepsilon_{ail}(\mathrm{mm}^{-1})$	$(1.45\pm0.09)\times10^{-3}$	$(1.67\pm0.09)\times10^{-3}$
STEP 2	$\varepsilon_{aink}(\mathrm{mm}^{-1})$	315.0±0.4	286.1±0.8
STEP 4	$\varepsilon'_{s2il}/\varepsilon'_{s1il}$	-0.27±0.05	-0.29±0.05
	$\varepsilon_{ail}(\mathrm{mm}^{-1})$	$(2.14\pm0.06)\times10^{-3}$	$(2.46\pm0.36)\times10^{-3}$
	$\varepsilon_{aH_2O}(\mathrm{mm}^{-1})$	$(2.77\pm0.01)\times10^{-3}$	$(3.56\pm0.02)\times10^{-3}$
STEP 5	$\varepsilon'_{s1il}(\mathrm{mm}^{-1})$	21.16±0.04	18.57±0.03
	$\varepsilon'_{s2il}(\mathrm{mm}^{-1})$	-5.8±0.9	-5.3±0.9
	$\varepsilon_{ail}(\mathrm{mm}^{-1})$	$(2.2\pm0.2)\times10^{-3}$	$(2.7\pm0.3)\times10^{-3}$
STEP 6	$\varepsilon_{aink}(\mathrm{mm}^{-1})$	319±1	289±2

4. Discussion and Conclusions

Intralipid 20% has been recently proposed as a first step towards a reference diffusive medium for tissue-simulating phantoms [17]. In this paper we have investigated the limits of applicability of the linear relationships between the optical properties of diluted Intralipid and the volume concentration. The validity of these linear relationships is commonly assumed when liquid tissue phantoms are prepared, but they are applicable only if the microphysical properties of scattering particles are not affected by dilution, and if the independent scattering approximation is fulfilled. The results presented in section 3 showed that the microphysical properties of suspended particles are not appreciably affected by the dilution in water or by the addition of India ink. As for the effect of dependent scattering experimental results showed that the absorption coefficient is not affected, while the reduced scattering efficiency slightly decreases when concentration increases. Deviations from linearity can be accounted for with a corrective term proportional to the square of the concentration. However, for concentrations of practical interest for tissue phantoms ($\rho_{il} < 0.1$ to obtain $\mu'_s < 2$ mm^{-1}) deviations remain within about 2%.

The effect of dependent scattering on the extinction coefficient has been investigated in many papers [19,20,24–26] but to our knowledge results on μ_a and μ'_s have been reported only in [20]. Measurements reported in Ref. [20] have been carried out at λ = 632.8 nm and were focussed on high concentrations of Intralipid 20% (up to ρ_{il} = 1). The results reported in this paper are in agreement with those of Ref. [20] both for μ_a and μ'_s: absorption is not affected by dependent scattering while the efficiency of reduced scattering decreases as ρ_{il} increases. From measurements at 751 and 833 nm for moderate concentrations ($\rho_{il} < 0.15$) we obtained for the ratio $\varepsilon'_{s2il}/\varepsilon'_{s1il}$ the values -0.27±0.04 and -0.29±0.05 respectively. The value obtained from Ref. [20] for λ = 632.8 nm and high concentrations was -0.37.

The results we obtained for ε_{eil}, ε_{ail}, and ε'_{sil} are in excellent agreement with the results we reported in Ref. [23] for different batchs of Intralipid 20%. For comparisons with other published data we refer to the discussion in Ref. [17].

The results presented in this paper show that the optical properties of dilutions of Intralipid can be predicted with high accuracy for all concentrations of practical interest for tissue phantoms once the parameters ε'_{s1il}, ε'_{s2il}, and ε_{ail} have been determined. The accuracy is ultimately limited by random and systematic errors on the parameters ε'_{s1il}, ε'_{s2il}, and ε_{ail} and on the concentration ρ_{il}. With our setup the parameters ε'_{s1il}, ε'_{s2il}, and ε_{ail} have been determined with a random error of 0.2%, 15%, and 10%, respectively. As for systematic errors, from the dis-

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cussion in Ref. [12] comes that the main contribution is due to the uncertainty in positioning the fibers and to the error on the absorption coefficient of water. The absorption coefficient of water has been measured with error of 0.5%. The corresponding systematic errors on $\varepsilon'_{s1\,il}$, $\varepsilon'_{s2\,il}$, and $\varepsilon_{a\,il}$ are of about 0.5%. In positioning the source and the receiving fibers we estimated an uncertainty of 0.1 mm. The corresponding systematic errors are of 1%, 1%, and 5% for $\varepsilon'_{s1\,il}$, $\varepsilon'_{s2\,il}$, and $\varepsilon_{a\,il}$ respectively, when measurements of fluence are carried at interfibre distances between 10 and 35 mm, as is the case for the results reported in section 3. However, this error can be easily reduced if measurements are carried out for larger interfibre distances. As an example, with an uncertainty of 0.1 mm the errors are reduced to 0.2%, 0.2%, and 1%, i.e., smaller than random errors, if measurements are carried out between 20 and 45 mm. The disadvantage in carrying out measurements at larger interfibre distances is the larger volume of diffusive medium necessary to mimic the infinite medium.

We point out that the intrinsic optical properties of Intralipid have been presented referring to the volume concentration ρ_{il}. Since the density of Intralipid 20% is close to the density of water, it is possible with negligible error to obtain the optical properties referred to the weight concentration ρ_{wil} taking into account that for concentrations of practical interest is $\rho_{il} \cong \rho_{wil}/0.988$.

In conclusion we have shown that by using the method of water absorption, and including the corrective term to take into account the effect of dependent scattering on the scattering properties, it is possible to characterize the reduced scattering coefficient of diluted Intralipid with an overall error of less than 1% on $\mu'_{s\,il}$, and that at the NIR wavelengths we investigated for dilutions of practical interest the absorption coefficient is practically equal to the absorption of pure water. Furthermore, using the calibrated Intralipid it is possible to measure the absorption coefficient of India ink with the same accuracy we have on the reduced scattering coefficient of Intralipid. Mixing calibrated Intralipid as a scattering medium and calibrated India ink as an absorbing medium it is therefore possible to obtain liquid phantoms with the desired scattering and absorption properties with errors smaller than 1%.

Also in the light of a recently published paper [11], it seems difficult to measure the optical properties of a solid phantom with accuracy similar to that obtained for the Intralipid and India ink phantoms. Liquid phantoms with well known optical properties can be therefore useful to investigate and to understand the ultimate accuracy obtainable with different techniques proposed for measuring the optical properties of diffusive media, and more in general for studying photon migration. Using black containers with small transparent windows for input and output of emitted and of received light it is possible to obtain homogeneous diffusive media with well defined and known boundary conditions whose optical properties can be easily adjusted changing the concentration of scatterers and of absorbers. Layered media can be also made by using thin foils of slightly scattering material to separate different layers [27]. Furthermore, inhomogeneities can be easily put inside the liquid to mimic the effect of abnormalities on photon migration. With liquid phantoms, being possible to repeat measurements before and after putting the inhomogeneity inside without changing anything else, also small perturbations on light propagation can be measured with high accuracy [28].

Acknowledgments

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