Measurement of Optical-Transport-Coefficients of Intralipid in Visible and NIR Range

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ABSTRACT

This article presents a modified method of measuring the optical-transport-coefficients of Intralipid: the diffuse reflectance of Intralipid with added-ink R_d^{ink} is measured by using an integrating sphere to calculate the scattering coefficient μ'_s, the effective attenuation coefficient μ_{eff} is measured by scanning the surface of pure Intralipid suspension with a cut-end, high NA fiber-optic tip (ϕ600μm, NA=0.48) in order to directly derive the absorption μ_a of pure Intralipid. In the same way, their wavelength dependencies between 0.48-0.85μm are measured by utilising Ar^+, Dye and Ti:Sapphire lasers.

Experiments show that $\mu_s(\lambda)$ varies with λ according to the previously reported Mie theory, $\mu'_s(\lambda)$ decreases with λ while $R_d^{ink}(\lambda)$ is nearly invariant within the wavelength range; the scattering anisotropy $g(\lambda)$ tends to decrease linearly with λ from 0.91 to 0.78; $\mu_a(\lambda)$ first decreases with λ till $\lambda \approx 0.61$μm and then gradually increases with λ. In the R_d^{ink} experiments, it has been found that when the port of the integrating sphere is lifted above the liquid surface, the dependence of the measured intensity with the height H can be well-fitted into an exponential relation for H≤3cm and Lorenzian relation for H≤10cm, so R_d^{ink} can be accurately derived by a simple extrapolation over a few measured points to H=0. Monte-Carlo simulation is applied to analyse the results.

1. INTRODUCTION

Light propagation in turbid biological tissues in NIR (therapeutic window within 0.6~1.3μm) has shown evergrowing interest for many medical applications[1]. In recent years, several models have been developed that can bridge over the difficulties in predicting the fluence rates $\phi(r, z)$ within tissue from the external quantities, i.e., the reflectance $R(r, \theta)$ and transmittance $T(r, \theta)$ of light by tissue[2,3]. However, as most of the models are derived on the basis of the so-called diffusion theory and the accuracy of these calculations depends to a great extent upon the accuracy of the optical parameters of tissue adopted for calculation, the inverse process, i.e., the measurement of optical properties of tissue in vitro and in vivo is certainly an essential problem in laser-tissue interaction.

Intralipid-10% is a colloidal suspension in which ultra-fine soybean oil drops are stably dispersed in water for parenteral nutrition. As Intralipid is highly scattering like milk with relatively low absorption in visible and NIR, it has been often used as an ideal tissue phantom medium to simulate light dosimetry in photodynamic therapy (PDT) and other laser-tissue effects when properly diluted and added with ink or dyes, therefore the determination of the optical properties of Intralipid is essential, especially when the layered structure samples for optical tomographic measurements are used. Normally, for turbid mediums, three independent measurements have to be made in order to derive the optical properties, i.e., the absorption coefficient μ_a, the scattering coefficient μ_s and the scattering anisotropy g. Although numerous measurements have been reported, significant discrepancy exists as a result of different methods of measurement and sample preparation[4]. Analysis by J. T. Allardice[5] on differences in the scattering coefficient μ_s of Intralipid from batch to batch reveals that the inconsistance in studies measuring μ_s might be resulted from unreliable sample preparation. This is because Intralipid is not specially designed for optical uses, differences in particle diameters and surfactant contents lead to different particle distributions (due to particle aggregation) and the aggregation may change with time and even concentration.

 0-8194-1429-8/94/$6.00

On the other hand, improper measurement procedures may also lead to significant uncertainty that can be ten times larger than the experimental errors, especially in the derivation of g and μ_a. An optimal method of measurement should therefore be chosen according to the sample optical properties. Recently, S. T. Flock, et. al., presented a simple and effective way by measuring the diffuse reflectance R_d^{ink} with added ink, i.e., known μ_a^{ink}. This method, however, is not very precise in deducing μ_a of pure Intralipid because R_d is insensitive to the reduced scattering to absorption coefficient $N'(=\mu'_s/\mu_a)$ for $R_d>0.9$. To overcome these difficulties, we modified the procedure as following: 1. measure the diffuse reflectance of Intralipid with ink R_d^{ink} by an integrating sphere to calculate the reduced scattering coefficient μ'_s; 2. measure the local diffuse reflectance $R_d(r)$ by scanning the surface of the pure Intralipid solution with a cut-end, high NA multimode fibre tip to derive the effective attenuation coefficient μ_{eff}, then μ_a of pure Intralipid can be easily calculated from μ'_s and μ_{eff}. In the same way, their wavelength dependencies between 0.48-0.85μm are measured by utilising Ar^+, Dye and Ti : Sapphire laser sources.

2. THEORY AND METHOD

10% stock solution of Intralipid is used as the scattering phantom medium which, in our experiments, is diluted in distilled water to adjust the scattering properties. The main contents of Intralipid® 10 Novum (Pfrimmer Kabi GmbH+Co., KG Erlangen, Germany) are listed below (per 1000ml):

Soybean oil: 100g,
Cholin (3-sn-Phosphatidy): 6g,
Glycerol: 22g;

Black ink (Pelikan, Germany) with volume concentration of about 0.05~0.1% is used to provide the required light absorption, i.e., the controllable μ_a^{ink} which has to be much larger than that of pure Intralipid. The reduced scattering coefficient of ink μ'^{ink}_s is approximated to be $4cm^{-1}$ and assumed to be wavelength independent[6].

2.1. Collimated transmittance

In turbid medium, light attenuation is caused not only by absorption but also by scattering. So, as has already been well established, a simple and forward way to determine the extinction or total attenuation coefficient μ_t ($\mu_t = \mu_a + \mu_s$) of the highly scattering medium like Intralipid is to measure the attenuation of the unperturbative, collimated light passing through the sample by a narrow beam or the so-called pin-hole optical technique. The geometry of such detection technique is shown in Fig.(1). Before incident on the sample cell, the laser beam is collimated and spatially

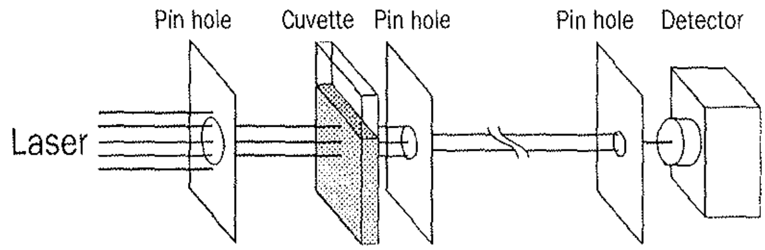


Fig.(1). Schematic of the experimental setup for collimated transmittance measurement.

filtered by the first pin-hole to reduce the beam divergence. The sample cells are standard fused-silica glass cuvettes with thickness of 0.1000cm and 1.000cm in which Intralipid dilutions are filled with volume concentration varying from 0.1% to 5%. A second ϕ2mm pin-hole is placed just behind the sample cell and a third ϕ0.45mm pin-hole is located 55cm away. This experimental arrangement provides the collimated transmittance measurement with a collecting aperture of solid angle smaller than 60μrad. The collimated intensity at the photo-detector I can be expressed as:

$$I/I_0 = e^{-\mu_t \cdot d} \tag{1}$$

where I_o is the measured intensity for distilled water which can be used to compensate the reflecting loss due to the refractive index mismatch at the boundary (assuming $n_{Intralipid} = n_{water}$). Here the scattering coefficient of the dilution μ_s is also assumed to the proportional to the volume concentration of Intralipid-10% in water.

In the experiments, we find

1). if the pinhole just behind the sample is too small, diffraction might cause problems (especially for water measurement);

2). measurement error can be effectively reduced by injecting the sample into the cell instead of removing the cuvette every time.

As the light absorption of Intralipid is negligibly small compared with scattering ($\mu_a << \mu_s$), this measurement directly determines the scattering coefficient μ_s of Intralipid.

2.2. Total diffuse reflectance

In tissue optics, light propagation in turbid media is normally analysed using the radiation transfer theory. If the medium is optically homogenous and semi-infinite, the diffusion approximation to the radiative transfer equation reveals that the total diffuse reflectance R_d can be expressed as a function of the dimensionless ratio of the reduced scattering coefficient to the absorption coefficient N' ($N'=\mu'_s/\mu_a$) and the mismatch in refractive index ($n= n_s/n_o$) at the boundary[7]:

$$R_d = R(N', n) \tag{2}$$

n_s, n_o represent the refractive indices of the scattering medium and the upper surrounding layer, respectively. In this study, the refractive index of Intralipid is assumed to be approximately equal to that of water (e.g., $n_s \approx 1.34$) and Monte-Carlo (M-C) technique is applied to model the dependence of the total diffuse reflectance R_d on the reduced scattering to absorption ratio N'.

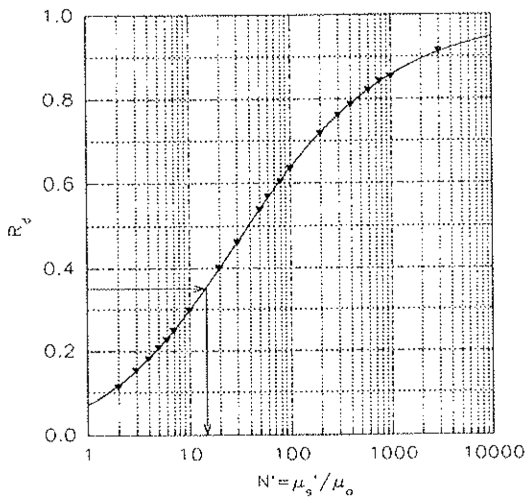


Fig.(2). M-C modeling of the relation of total diffuse reflectance R_d to the dimensionless ratio N'.

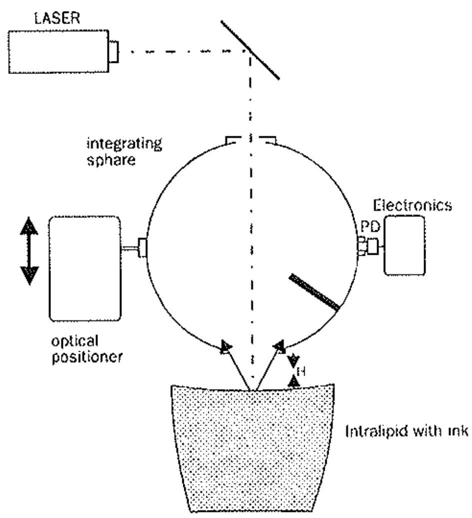


Fig.(3). Experimental setup for diffuse reflectance measurement.

As shown in Fig.(2), cubic spline interpolation is applied to the results of the M-C calculation while the solid points for $R_d \geq 0.9$ are calculated according to the similarity relation[8] because the straightforward M-C modelling in this case is extremely time-consuming. This one-to-one mapping in Fig.(2) for N' in terms of R_d measurement provides the second relation for the determination of μ'_s and g. If the absorption coefficient μ_a is known by adding black ink or dye ($\mu_a << \mu_a^{ink}$), then this measurement determines μ'_s as well as g ($g=1-\mu'_s/\mu_s$) of Intralipid.

Fig.(3) is the experimental setup for R_d^{ink} measurement. The collimated laser beam with a diameter less than 1.5mm is delivered to a 350ml cube-sample. In contrast to the previously reported 'point' radiance detection from a calibrated radiometer, the present system makes use of an integrating sphere which provides a more accurate approach for R_d detection. The port at the bottom of the integrating sphere which is used to collect the diffuse reflectance is 25mm in diameter and a small pork on the top provides an optical path which guides the incident laser beam to the sample as well as releases the specularly reflected beam R_{sp} from the sphere.

To avoid the boundary complication which may be induced from the insertion of a mylar film between the air/Intralipid surface, the integrating sphere is mounted on an optical positioner. Measuring the optical intensity $I_r(H)$ collected by the sphere at different height H, the reflected intensity I_d^{ink} can be derived by a simple extrapolation over a few (~3) points to H=0. This I_d^{ink} is then calibrated with I_d^{std} from a standard reflecting plate ($R^{std} \approx 99.6\%$) to derive the diffuse reflectance R_d^{ink} ($= I_d^{ink} \cdot R^{std}/I_d^{std}$).

2.3. Local diffuse reflectance

In principle, the third coefficient μ_a can also be determined through the above integrating sphere measurement of R_d (without added ink). However, as for pure Intralipid, the total diffuse reflectance R_d falls into the range ($R_d \geq 0.90$) where a slight experimental error in R_d measurement induces relatively large errors in deriving μ_a, as shown in Fig.(2).

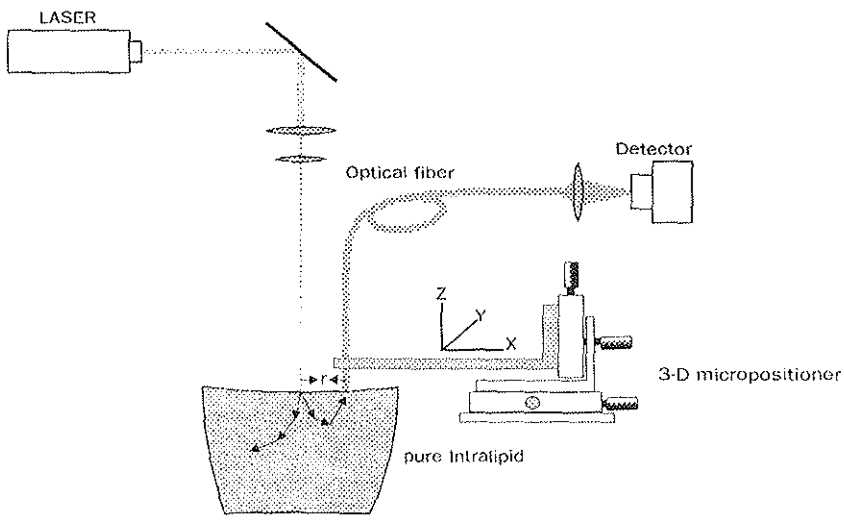


Fig.(4). Experimental setup for local reflectance measurement

In order to overcome these difficulties, the local diffuse reflectance $R_d(r)$ was measured as a more accurate approach due to the fact that the fluence rate is strongly dependent on the absorption coefficient μ_a (see Eq.(4)) and moreover the high N' of pure Intralipid provides enough spatial resolution for the $R_d(r)$ detection even with a 600μm-fiber-optic tip. For the cylindrically symmetric geometry of a semi-finite medium with collimated, narrow beam incidence, the analytical solution to the diffusion equation is used as the mathematical model to determine μ_a from the surface fluence rate measurement which describes the diffuse reflectance at r ($r >> 1/\mu'_s$) from the point source as[9]:

$$R(r)= K_0 \frac{e^{-\mu_{eff} r}}{r^2} \tag{3}$$

where K_0 is a constant which depends on the source-detector geometry. The effective attenuation coefficient μ_{eff} is defined as:

$$\mu_{eff} = \sqrt{3\mu_a(\mu_a + \mu_s')} \tag{4}$$

Fig.(4) shows the experimental arrangement for the fluence rate measurement. The laser beam is collimated to within ϕ0.4mm and then delivered down onto the surface of the pure Intralipid sample which is filled in a large beaker (600ml). A high NA (NA=0.48), end-cut optical fiber (ϕ600µm silica core/plastic cladding) is mounted on a 3-D Precision positioner. During the measurement, the fiber-optic tip is adjusted just touching the liquid and scanned from $r \approx 0.8$mm to $r \approx 10$mm. As μ'_s is already known from the R_d measurement and μ_a is negligibly small compared with μ'_s, i.e., $\mu_{eff} \approx (3\mu_a\mu'_s)^{1/2}$, then Eqs.(3) and (4) relate the absorption coefficient μ_a directly to the local diffuse reflectance rate $R_d(r)$. μ_a can be obtained by fitting the slope of $\ln[r^2 \cdot R(r)]\sim r$ at the known position r to the measured intensities with μ_{eff} as free parameter.

3. RESULTS AND DISCUSSION

3.1. Evaluation of the experimental results (He-Ne laser λ = 632.8nm):

For the 10%-Intralipid suspension which is used in our experiments, variations in μ_t from batch to batch are negligibly small compared with from different sample preparations. Fig.(5) shows the measured results from different dilutions (0.05%~2.5%) of Intralipid-10% suspensions. For the samples with concentration C lower than 0.3%, the measured μ_t is much higher those with higher concentration and increases with decreasing concentration, although the attenuation lengths $CL\mu_t$ of these samples are longer than those with higher concentration C but measured in a thinner cuvette (L=0.1cm). This phenomenon may be explained by the fact that Intralipid like most tissues is a highly forward scattering medium (g≥0.85). The lower the reduced scattering coefficient μ'_s (i.e., μ_s or C decreases and g increases)

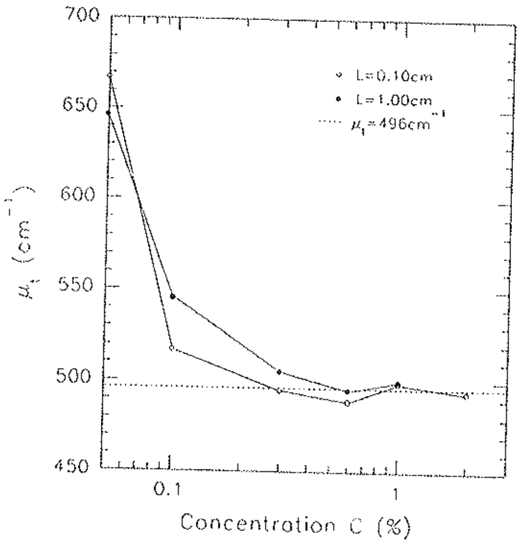


Fig.(5). The variation of μ_t measured with different Intralipid-10% dilutions.
(L is the thickness of the standard cuvette.)
(μ_t refers to the scattering coefficient of pure Intralipid)

the more the least scattered or ballistic photons will be collected by the pinhole detector as the unperturbative collimated transmittance. On the other hand, Intralipid as a colloidal suspension, the dynamic aggregation equilibrium (m-mer$\Leftrightarrow$(m+1)-mer−1) can be changed by dilution which might cause a slight deviation of the linear $\mu_t \propto C$ relation and a decrease of the scattering anisotropy g. When C decreases, a small portion of large aggregates might be splitted into small aggregates, the particle size distribution deviates toward more Rayleigh scatterers, so the concentration of the scatterers may be slightly enhanced and the scattering anisotropy g may be slightly decreased. Accordingly this effect will lead to a large measured μ_t. The pinhole just behind the sample cell can not be too small, otherwise diffraction may cause the scattered beam reentering the pin-hole detector. The fluctuation of μ_t for samples within large concentration C is mainly induced by inaccurate dilution during sample preparations. Based upon the above reasons, samples with $C > 0.3\%$ are adopted in our later experiments, which reduces the experimental error to within ± 4% limited by the inability of discriminating the least forward scattering from the unscattered, collimated light and by the detection sensitivity.

The radial distribution of the diffuse reflectance $R_d(r)$~r which is calculated by the inverse M-C technique with the measured coefficients (μ_s=496cm^{-1}, μ_a=6.6cm^{-1}, g=0.85) verifies that the leak-out of diffuse reflectance R_d^{ink} when measured with a ϕ25mm-port integrating sphere is in principle negligible (<1%), whilst in experiments the standard error for R_d^{ink} measurement is no more than ±2.9%. Therefore by using integrating sphere, the reduced scattering coefficient μ'_s can be measured with an improved accuracy of about ±5.6%. However, as the solvability of ink drops in Intralipid is not as stable as in water, it has been found that some ink particles may either segregate or soak above the liquid surface acting as an 'absorbing layer' which traps the reflected photons and accordingly results in a decrease (~6%) in R_d^{ink} measurements. Using the laser dyes instead of ink better phantom stability should be achieved.

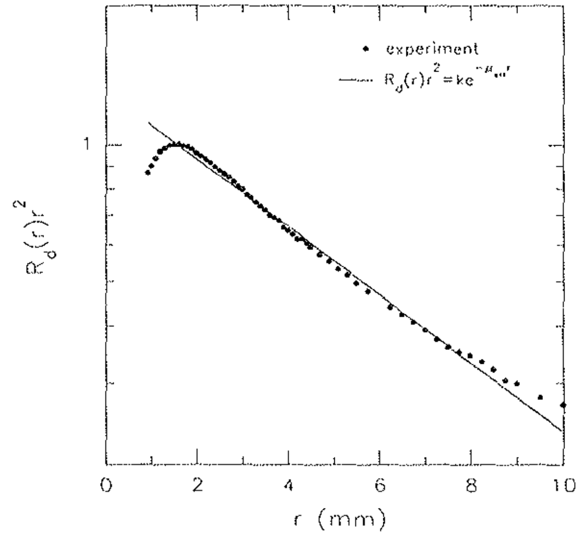


Fig.(6). Measured reflectance rate using a high NA cut-end fiber optical detector.

Fig.(6) shows the experimental results for fluence rate measurements. The solid line is plotted by fitting the data to Eq.(3). In this way μ_{eff} is derived: μ_{eff} = 0.1639mm^{-1}(Curve-fit starts at r_0=3mm). Possible experimental uncertainty may be caused by the following factors: 1. Eq.(3) is derived on the assumption of infinite narrow beam incidence while the practical fluence rate $R_d(r)$ is the convolution over the finite beam profile. This reduces the correlativity for the curve-fit (~6% for r_0>2mm); 2. the derived μ_{eff} changes with different starting point r_0 for curve-fit. 3. the measured fluence at large distance (r>8mm) may be overestimated as a result of background noise, finite boundary and fluorescent light from the Intralipid itself[10].

To summarize the above analysis, the optical transport coefficients of Intralipid measured at λ=632.8nm are:

$\mu_s = 496\text{cm}^{-1} \pm 4\%$; $g = 0.853 \pm 2.5\%$; $\mu_a = 0.013\text{cm}^{-1} \pm 17.5\%$.

3.2. The dependence of the measured R_d^{ink} on the height H above the liquid surface:

The inner coating of the integrating sphere ($BaSO_4$ coated) is sensitive to liquid, direct contact with Intralipid especially when added with ink or dye may cause deterioration of the high reflectivity of the coatings, while the insertion of a thin mylar film may add possible boundary complications: 1.difficulty in the inverse calculation of N' from R_d; 2. the film becomes concave under the liquid pressure. This experiment was therefore designed to seek a simple relation for the dependence of R_d^{ink}. As shown in Fig.(7), the measured R_d^{ink}~H curve can be well-fitted into an exponential relation for H≤3cm:

$$I_d^{ink}(H) = I_d^{ink}(H=0)\cdot e^{-\alpha H} \tag{5}$$

where α is a constant which is dependent on the source-detector geometry and the optical properties of the sample. Further detailed experiments show that this relation is valid over a wide range with R_d^{ink} varying from 0.2 to 0.94 (for pure Intralipid). If α is plotted vs. different $R_d(H=0)$, the least square fit shows a linear relation as shown in the inset of Fig.(7). This interesting result is worthy of further investigation.

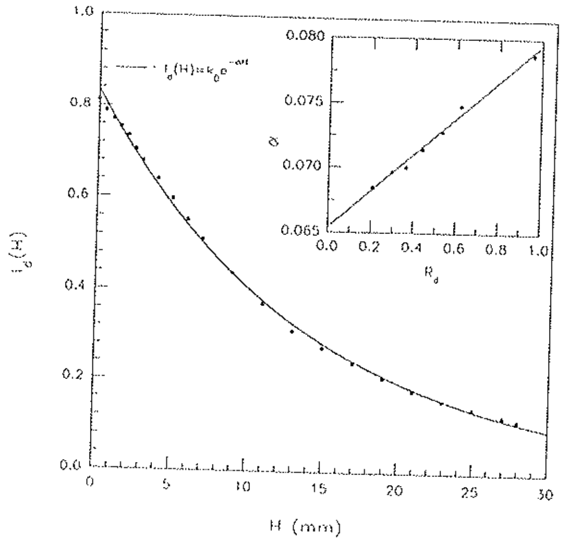


Fig.(7). The reflected intensity I_d with height for H≤3cm.

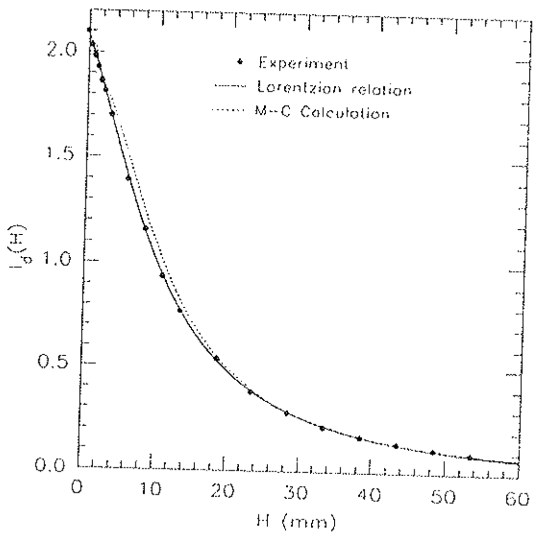


Fig.(8). The reflected intensity I_d with height for H≤6cm.

If the experiment is extended over a wider range to H≤6cm, the experimental curve can be well-fitted into Lorentzian function:

$$I_d^{ink}(H) = a + b/(1+((H-c)/H_0)^2) \tag{6}$$

where a, b, c, H_0 are constants. This equation seems to be reasonable because when $H\to\infty$, Eq.(6) can be approximated to the relation $R_d^{ink} \propto H^{-2}$ which is in agreement with the radiative transfer law. This R_d^{ink}~H relation can also be calculated by the following integration:

$$I_d^{ink}(H) = \int_0^{\infty}\int_0^{R_0}\int_0^{\infty}\int_0^{2\pi} \frac{R(r,\varphi)}{(H^2+r^2+r_0^2+2rr_0\cos\phi)^{3/2}} Hrr_0\,dr\,dr_0\,d\phi\,d\phi' \tag{7}$$

where $\varphi = \cos^{-1}(H/(H^2 + r_0^2 + r^2 + 2rr_0\cos\phi)^{1/2})$, $\phi = \phi_1 - \phi_2$. R(r, φ) is the spatial distribution of diffuse reflectance which is calculated from M-C modelling. The result is shown in Fig.(8) which coincides with experiment.

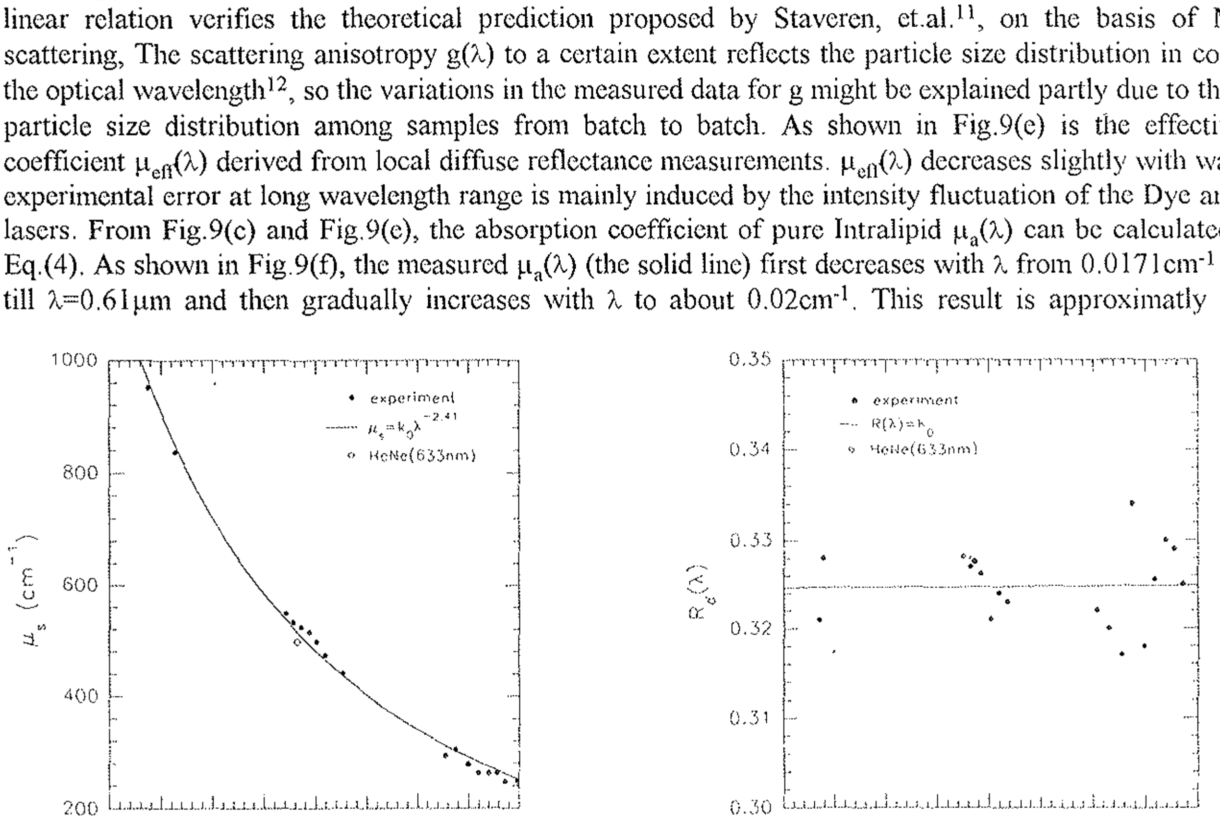
3.3. Wavelength dependencies of μ_s, μ_a, g from 0.48~0.85μm:

The wavelength dependencies of the optical properties of Intralipid μ_a, μ_s, g are measured in the same way as described above in section 2 by ultilizing:

Ar$^+$ laser (Spectra-Physics, Model 2035, λ=488nm, 514.5nm),
Dye laser (Spectra-Physics, Model 375B, λ=615~714nm),
Ti:Sapphire laser (Spectra-Physics, Model 3900S, λ=720~850nm).

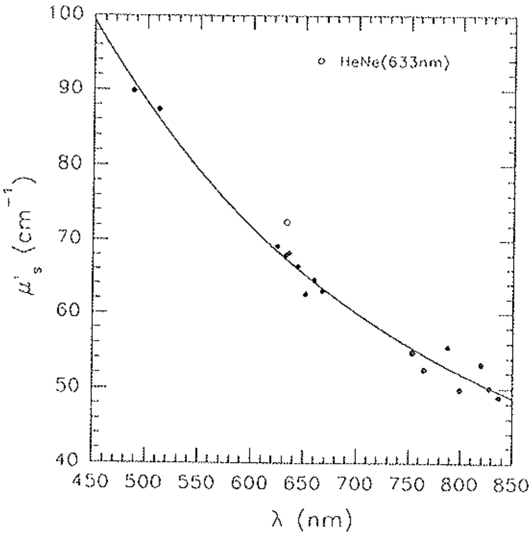
The combination of these three lasers makes it possible to provide more detailed data than the previously reported study over this wide wavelength range. Figs.(9)(a)~(f) are the measured results.

A quantitative analysis of Figs.(9) shows that the scattering coefficient $\mu_s(\lambda)$ decreases with λ according to Mie theory, i. e., $\mu_s(\lambda) \propto \lambda^{-m}$ (m=2.41), as shown in Fig.9(a) which is consistent with the previously reported results by Staveren, et al.[11], (m=2.4) and Flock, et al.[6], (m=2.33). The diffuse reflectance with added ink $R_d(\lambda)$ is nearly invariant with λ within the wavelength range of our measurements as shown in Fig.9(b) because both $\mu_s(\lambda)$ and $\mu_a(\lambda)$ of black ink decrease with wavelength. By combining these results $\mu_s(\lambda)$~λ and $\mu_a(\lambda)$~λ of ink the reduced scattering coefficient of Intralipid $\mu'_s(\lambda)$ can be derived and is found to decrease with λ as shown in Fig.9(c); The scattering anisotropy g(λ) calculated from Figs.9(a),(c) tends to decreases linearly with λ from 0.91 to 0.79. This well-fitted linear relation verifies the theoretical prediction proposed by Staveren, et.al.[11], on the basis of Mie theory of scattering, The scattering anisotropy g(λ) to a certain extent reflects the particle size distribution in comparison with the optical wavelength[12], so the variations in the measured data for g might be explained partly due to the difference of particle size distribution among samples from batch to batch. As shown in Fig.9(e) is the effective attenuation coefficient $\mu_{eff}(\lambda)$ derived from local diffuse reflectance measurements. $\mu_{eff}(\lambda)$ decreases slightly with wavelength. The experimental error at long wavelength range is mainly induced by the intensity fluctuation of the Dye and Ti:Sapphire lasers. From Fig.9(c) and Fig.9(e), the absorption coefficient of pure Intralipid $\mu_a(\lambda)$ can be calculated according to Eq.(4). As shown in Fig.9(f), the measured $\mu_a(\lambda)$ (the solid line) first decreases with λ from 0.0171cm^{-1} to 0.0135cm^{-1} till λ=0.61μm and then gradually increases with λ to about 0.02cm^{-1}. This result is approximatly in consistance

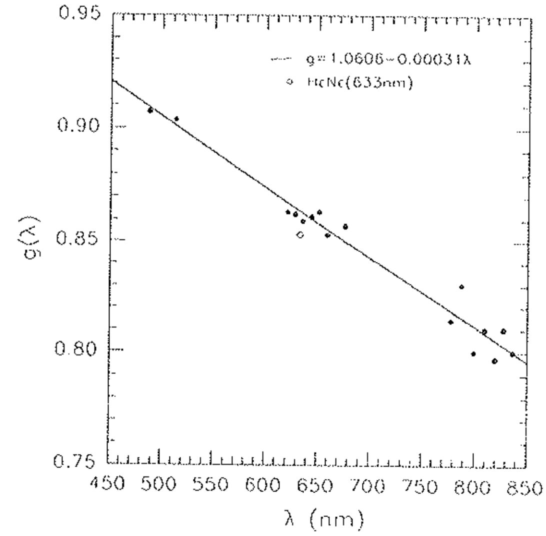


(a). Scattering coefficient $\mu_s(\lambda)$.

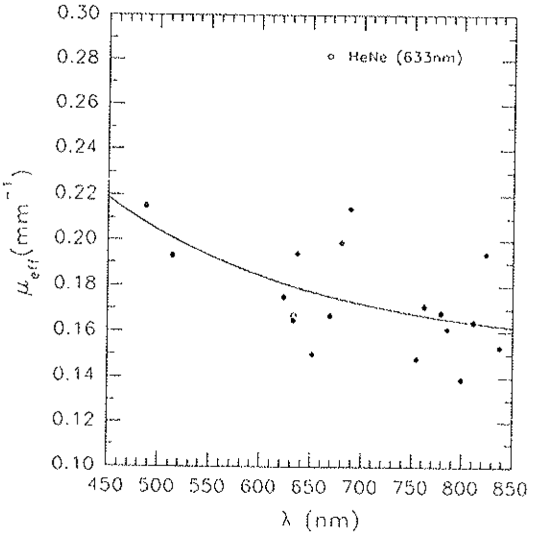
(b). Total diffuse reflectance $R_d(\lambda)$.



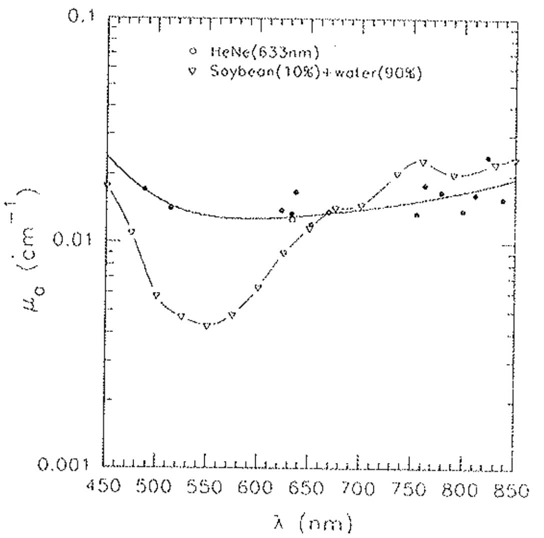
(c). Reduced scattering coefficient $\mu'_s(\lambda)$.



(d). Scattering anisotropy $g(\lambda)$.



(e). Effective attenuation coefficient $\mu_{eff}(\lambda)$.



(f). Absorption coefficient $\mu_a(\lambda)$.

Fig.(9). The wavelength dependences of the optical properties of Intralipid-10% from 488nm to 850nm.

with the combined contributions of soybean oil and water. The dash line is the calculated absorption coefficient of Intralipid-10% by contents, i.e., soybean oil (10%) + water(90%), based upon the previously reported measurements for soybean oil and distilled water[6,13,14]. For short wavelength range ($\lambda<0.55\mu m$) the absorption of water is negligibly small compared with soybean oil ($\mu_a \approx 0.01 cm^{-1}$); then as the absorption coefficient of soybean oil decreases with λ both contents contribute to μ_a; in the long wavelength range with $\lambda>0.7\mu m$, water turns to the main absorbing source of the mixture. Except the valley between 0.5 and 0.6μm, the measured curve for $\mu_a(\lambda)$ of Intralipid coincides with the calculated curve. In the above analysis, the absorption of choline is ignored.

4. CONCLUSIONS

In this paper we have developed a combined method for the measurement of optical transport coefficients of Intralipid. The diffuse reflectance with added-ink is measured by using an integrating sphere to derive reduced scattering coefficient μ'_s and the scattering anisotropy g; The absorption coefficient μ_a is calculated from the effective attenuation coefficient μ_{eff} which is measured by mapping the reflectance rate $R_d(r)$ of pure Intralipild with a cut-end, high NA fiber. Experiments show that this simple method can provide an improved accuracy for g and μ_a to within 2.5% and 17.5% respectively. The measured results for λ=633nm are μ_s = 496cm^{-1}, g = 0.853, μ_a = 0.013cm^{-1}. Their wavelength dependencies are measured in the same way by utilising Ar^+, Dye and Ti:Sapphire lasers. The detailed experiments over this wide wavelength range from 488 to 850nm verify the previously reported results by Staveren et.al.[11] on the basis of Mie theory which predicted: $\mu_s(\lambda) \propto \lambda^{-m}$ (m=2.41), $g(\lambda)$ decreases linearly with (λ). The diffuse reflectance when measured with an integrating sphere can be accurately derived by a simple extrapolation over a few measured points to H=0 (H is the height of the pork of integrating sphere above the liquid surface).

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