Title
Absolute measurement of absorption and scattering coefficients spectra of a multiply scattering medium

Authors
Fantini, Sergio
Franceschini, Maria-Angela
Fishkin, Joshua B
et al.

Publication Date
1994-07-28

DOI
10.1117/12.180732

Absolute measurement of absorption and scattering coefficients spectra of a multiply scattering medium

Sergio Fantini*, Maria Angela Franceschini*, Joshua B. Fishkin, and Enrico Gratton

Laboratory for Fluorescence Dynamics,
Department of Physics, University of Illinois at Urbana-Champaign,
1110 West Green Street, Urbana, Illinois 61801-3080

*Permanent address: Istituto di Elettronica Quantistica-Consiglio Nazionale delle Ricerche,
Via Panciatichi, 56/30, 50127 Firenze, Italy

ABSTRACT

On the basis of the diffusion theory model, frequency-domain spectroscopy allows for a quantitative determination of the absorption (μ_a) and scattering (μ_s') coefficient spectra of a homogeneous multiply scattering medium. We performed measurements using an intensity modulated light emitting diode (LED) as the light source. The LED's spectral distribution permits the study of a spectral region extending for about 80 nm. Data sets (phase shift and average intensity) at two different source-detector distances are acquired: the absorption and scattering coefficient spectra of the medium are then calculated from analytical expressions for μ_a and μ_s'. Methylene blue (peak absorption wavelength 656 nm) is used as a test absorbing material. The methylene blue is dissolved in an aqueous Liposyn solution which serves as the multiply scattering medium. The relative amounts of absorber and scatterer are chosen such that the values of μ_a and μ_s' match typical values in tissues. The results obtained for $\mu_a(\lambda)$ with this LED based technique are in quantitative agreement with those obtained with a standard spectrophotometer in a non-scattering regime.

1. INTRODUCTION

The determination of the optical properties of tissues is important in many fields of medicine, both for tissue diagnostics and the monitoring of physiologically important processes.[1,2,3,4,5] In particular, the measurement of the concentration of a chromophore in biological tissues can provide useful information. A spectroscopic study of tissues is complicated by the fact that they are multiply scattering media, and thus the attenuation of light is due both to absorption and scattering. The problem of separating the effects of absorption and scattering has been treated using steady state[6,7,8] and time resolved techniques (in both the time-domain[9] and frequency-domain[10,11]). We have performed measurements in the frequency-domain, in which the intensity of the light source is modulated at radio frequency (typically at tens to hundreds of megahertz), and the detected quantities are the average intensity (DC component), the amplitude of the intensity oscillation (AC component), and the phase shift of the detected light relative to the exciting signal (Φ). A model based on the diffusion approximation to the Boltzmann transport equation has been developed.[12] It provides expressions for the measured quantities (DC, AC, Φ) in terms of the optical parameters of the medium, which are the absorption coefficient (μ_a), the transport scattering coefficient (μ_s') and the index of refraction (n). This model is valid for a homogeneous medium in a multiply scattering regime and far from sources or boundaries. We used a light emitting diode (LED) as the light source, and we developed an experimental protocol, based on a two-distances measurement, which allows for the determination of μ_a and μ_s' from analytical expressions. The measurable ranges of μ_a and μ_s', as well as the sensitivity of this method to changes in μ_a and μ_s', are discussed.

0-8194-1424-7/94/$6.00

2. METHODS

2.1. Experimental setup

The medium we have studied is an aqueous solution of Liposyn III 20% (an intravenous fat emulsion) from Abbott Laboratories, in which a test absorbing material (methylene blue) was dissolved. The concentration of Liposyn, which provided the scattering material, was chosen to be 7.7% by volume (i.e. solids content of 1.54%), yielding a medium scattering coefficient on the order of 20 cm^{-1}, which is typical for soft tissues.[13] Methylene blue has an absorption spectrum peaked at 656 nm and an extinction coefficient of 182 ± 1 $cm^{-1}mM^{-1}$ at 664 nm.[14] We used several concentrations of methylene blue, from 0 to 0.450 M, in order to cover a typical range of values of μ_a in tissues (0.01 to 0.1 cm^{-1}).[13] The measurements were conducted in a 2.3 *l* cylindrical container whose dimensions were 9 cm in height and 18 cm in diameter.

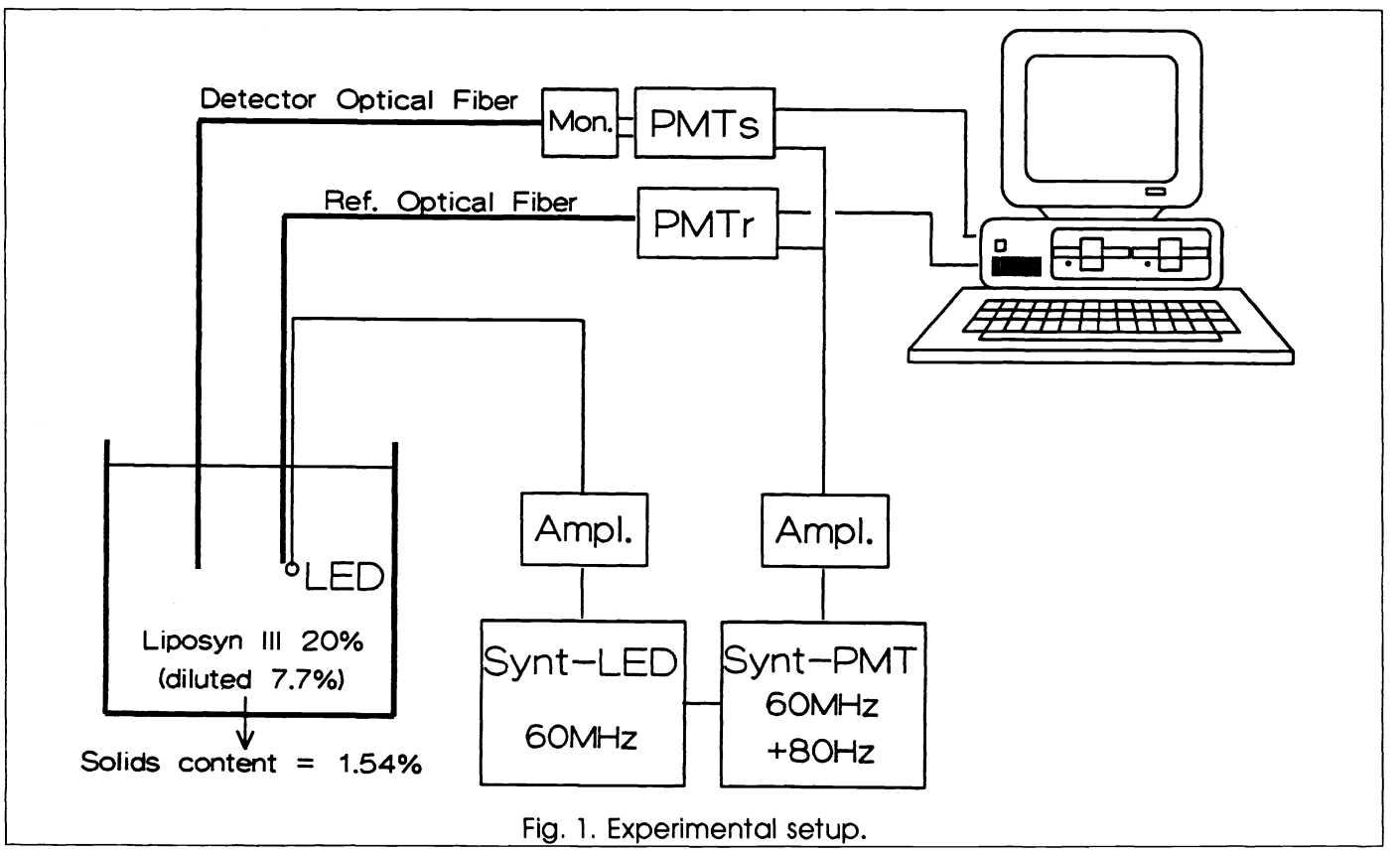

Fig. 1. Experimental setup.

As the light source, we utilized a light emitting diode (LED) manufactured by Hewlett Packard (part number: HLMP-4101). We modulated the LED's intensity by applying a sinusoidal voltage generated by a frequency synthesizer (Marconi Instruments, Model 2022A) and amplified by an ENI Model 403 LA RF Amplifier. In general, a frequency-domain measurement is more accurate at higher modulation frequencies, so we looked for the highest frequency of the sinusoidal voltage supply which provided a good AC signal at the LED's output. This frequency was 60 MHz for the LED we used, and the peak to peak supplied voltage was 10 V. The negative front of the sinusoidal voltage was less than the reverse breakdown voltage of the LED (15 V). In these working conditions, the LED's output was characterized by a peak wavelength of 665 nm, a spectral line fullwidth at half maximum of about 30 nm, a total power of about

0.2 mW, and a modulation ratio (AC/DC) of 60%. Its spectral emission enabled us to accomplish measurements at wavelengths ranging from 620 to 700 nm, which is appropriate for measuring the spectrum of methylene blue (λ_{max}=656 nm).

The multiply scattered light was detected by a bundle of glass optical fibers of overall diameter of 3 mm. This bundle was positioned at a distance r from the light source, and its output was dispersed via a monochromator (10 cm ISA Instrument SA, Inc.) and detected by the sample photomultiplier tube (PMTs). The spectral resolution was 4 nm. The detection system is a typical one for frequency-domain measurements, and is based on a well established cross-correlation technique.[15,16] The cross-correlation frequency was set to 80 Hz. We referenced the values of DC, AC, and phase detected by the sample PMT (PMTs) to those detected by the reference PMT (PMTr) which collected a signal from an optical fiber in close contact with the LED (both PMT's are manufactured by Hamamatsu (Japan), model R928). This procedure provided a correction for variations in the LED's output characteristics that might have occured during the measurement. The experimental setup is shown in Fig. 1.

2.2. Measurement protocol

The diffusion approximation to the Boltzmann transport equation provides analytical expressions for the frequency-domain parameters as a function of the absorption coefficient (μ_a), the transport scattering coefficient (μ_s'), and the index of refraction (n) of the medium.[12] These expressions, which are valid for a homogeneous infinite medium at points which are far from sources and boundaries, predict a linear dependence of Φ, $\ln(rU_{DC})$ and $\ln(rU_{AC})$ on r (where r is the source-detector separation, U_{DC} is the DC component of the photon density, U_{AC} is the amplitude of the AC component of the photon density). If we consider two different source detector separations, let's say r_1 and r_2 with $r_1 < r_2$, we can define the following quantities:

$$\rho = r_2 - r_1$$

$$\delta = \ln(r_2 U_{DC}(r_2)) - \ln(r_1 U_{DC}(r_1))$$

$$\alpha = \ln(r_2 U_{AC}(r_2)) - \ln(r_1 U_{AC}(r_1))$$

$$\varphi = \Phi(r_2) - \Phi(r_1).$$

Finally, making the further assumption that $\omega/2\pi << c\mu_s'/n$ (where $\omega/2\pi$ is the modulation frequency, and c is the speed of light in vacuum) we can write[12]:

$$\delta = -\rho\sqrt{3\mu_a(\mu_a + \mu_s')} \tag{1}$$

$$\alpha = -\rho\sqrt{\frac{3}{2}\mu_a(\mu_a + \mu_s')}\left(\sqrt{1 + \left(\frac{\omega n}{c\mu_a}\right)^2} + 1\right)^{1/2} \tag{2}$$

$$\varphi = \rho\sqrt{\frac{3}{2}\mu_a(\mu_a + \mu_s')}\left(\sqrt{1 + \left(\frac{\omega n}{c\mu_a}\right)^2} - 1\right)^{1/2} \tag{3}$$

We observe that the condition $\omega/2\pi << c\mu_s'/n$ holds for most biological tissues in the red-near IR spectral region for modulation frequencies up to 1 GHz. The idea is then to perform two measurements at two different source-detector separations (r_1 and r_2) from which to obtain δ, α, φ. We point out that the source terms do not appear in Eqs. 1-3, so that δ, α, φ depend only on the modulation frequency and on the optical parameters of the medium. Furthermore, the spectral instrument response factor cancels in δ and α, because they contain the ratio of U_{DC} and U_{AC} measured at different source-detector separations, but at the same wavelength. Once δ, α, φ are measured, μ_a and μ_s' can be calculated from Eqs. 1-3. Inspection of these equations shows that they are not independent, since they contain only two unknowns, i.e. μ_a/n and $n(\mu_a+\mu_s')$. If n is known, one can choose to obtain μ_a and μ_s' from three different sets of measurements: δ and φ (DC and phase), α and φ (AC and phase), δ and α (DC and AC). The analytical expressions for μ_a and μ_s' in the three cases are given in Table 1.

TABLE 1	δ, φ scheme (using DC and phase)	α, φ scheme (using AC and phase)	δ, α scheme (using DC and AC)
μ_a	$-\frac{\omega n}{2c}\frac{\delta}{\varphi}\left(\frac{\varphi^2}{\delta^2}+1\right)^{-\frac{1}{2}}$	$\frac{\omega n}{2c}\left(\frac{\varphi}{\alpha}-\frac{\alpha}{\varphi}\right)$	$\frac{\omega n}{2c}\frac{\delta}{\alpha}\left(\frac{\alpha^2}{\delta^2}-1\right)^{-\frac{1}{2}}$
μ_s'	$\frac{\delta^2}{3\mu_a\rho^2}-\mu_a$	$\frac{\alpha^2-\varphi^2}{3\mu_a\rho^2}-\mu_a$	$\frac{\delta^2}{3\mu_a\rho^2}-\mu_a$

On the basis of these expressions, the errors on μ_a and μ_s' can be evaluated from the estimates of the errors on δ, α, φ. The result is that, for typical values of the parameters in tissues, the errors on μ_a and μ_s' in the (δ, α) measurement scheme are much larger than those in the (δ, φ) and (α, φ) schemes. This is due to the fact that the values of δ and α are very close, differing by no more than a few percent. We chose to use the (δ, φ) scheme because the error on the DC signal is typically smaller than the error on the AC signal. The use of DC and phase in frequency-domain measurements was also suggested as an alternative to a phase and modulation (AC/DC) measurement.[10]

In summary, our measurement protocol consists of acquiring data at two source-detector distances (r_1, r_2) and calculating δ and φ. From the expressions relative to the (δ, φ) measurement scheme in Table 1, we obtain the values of μ_a and μ_s'. This procedure, applied wavelength by wavelength within the whole spectral region covered by the LED (620 to 700 nm), provides the spectra of μ_a and μ_s'. The choice of the values of r_1 and r_2 ($r_1 < r_2$) relies on two considerations:

- r_1 must be greater than the photon mean free path in order for Eqs. 1-3 to be valid;
- r_2 must allow for an acceptable detected light signal: since the density of photons decays exponentially with distance, this condition constitutes an upper limit for r_2.

We chose r_1 to be 1.5 cm, while r_2 ranged from 4.0 to 2.5 cm depending on the amount of absorber in the medium.

3. RESULTS

First we performed a control measurement on the aqueous Liposyn solution alone. In the absence of methylene blue we expect to measure essentially the absorption spectrum of water, while the scattering spectrum is related to the suspended particles of Liposyn. In Fig. 2 we show the spectra we measured for μ_a and μ_s'. The spectrum of μ_a is compared to values of μ_a for water measured at several wavelengths by Hale and Querry.[17] The order of magnitude of μ_a is the same for both quantities and their spectral dependence is qualitatively comparable. We attribute the deviations from the literature values of μ_a for water to a contribution of the Liposyn to absorption, and to boundary effects due to the finite size of the container we used. The spectrum of μ_s' is compared to the curve $\mu_s'(\lambda)$ predicted by van Staveren *et al.* on the basis of Mie theory calculations for 1.54% Intralipid.[18] Even if van Staveren considered a slightly different medium, we observe that the values of μ_s' and the spectral dependence are essentially reproduced.

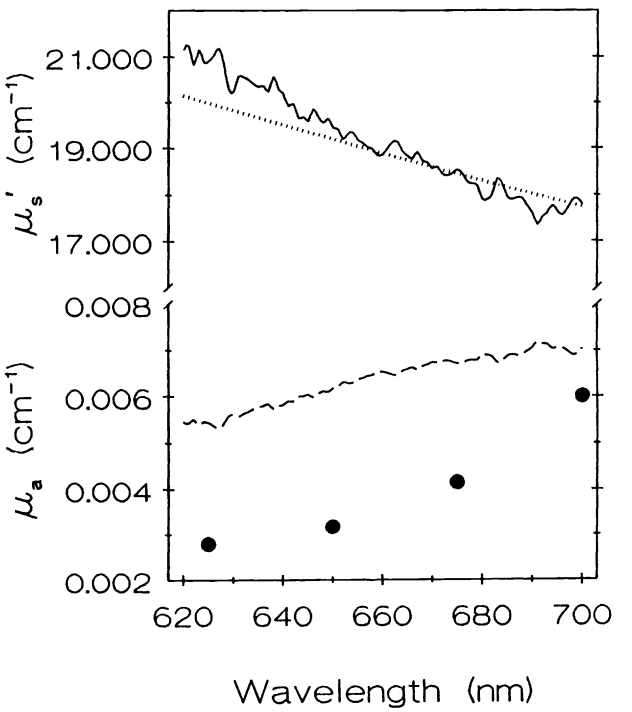

Fig. 2. Scattering (solid line) and absorption (dashed line) coefficients spectra of the Liposyn solution containing no methylene blue. The two spectra are compared, respectively, with $\mu_s'(\lambda)$ predicted by van Staveren *et al.* (dotted line) and with several values of $\mu_a(\lambda)$ for water known in literature (symbols).

Next we measured the absorption spectra for different concentrations of methylene blue in the Liposyn solution. We subtracted the absorption spectrum of the Liposyn solution containing no methylene blue to obtain the corrected absorption spectra for methylene blue alone. The results obtained with our measurement protocol were then compared to the absorption spectra obtained with a spectrophotometer (Perkin Elmer LAMBDA 5) for the same concentrations of methylene blue in a non-scattering solution. The comparison, reported in Fig. 3 for three methylene blue concentrations, shows a quantitative agreement between the measurements in the multiply scattering regime (performed via the frequency-domain LED technique) and the measurements in minimal scattering regime (performed by a standard spectrophotometer). We have also analyzed the dependence of μ_a and μ_s' on methylene blue concentration. The result is shown in Fig. 4 for $\lambda = 680$ nm: μ_s' is essentially unaffected by the concentration of absorber, while μ_a shows a linear dependence. We observe that our results have been obtained by assuming that $n = 1.33$ (which is the index of refraction of water in the spectral region considered). The good quantitative results we found justify such a choice for n.

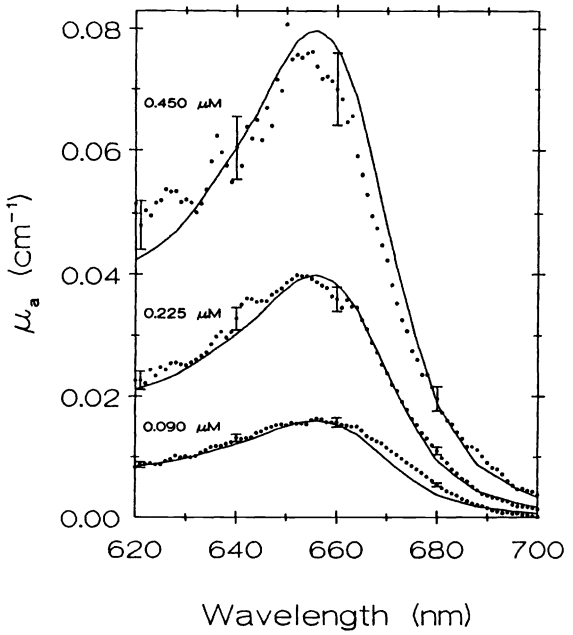

Fig. 3. Quantitative comparison between methylene blue (MB) absorption coefficient spectra measured in the strongly scattering medium by the LED technique (symbols) and in a non-scattering regime by a spectrophotometer (lines). The spectra correspond to MB concentrations of 0.090, 0.225, and 0.450 μM as labeled. Errors bars for the experimental data relative to the strongly scattering medium are shown every 20 nm.

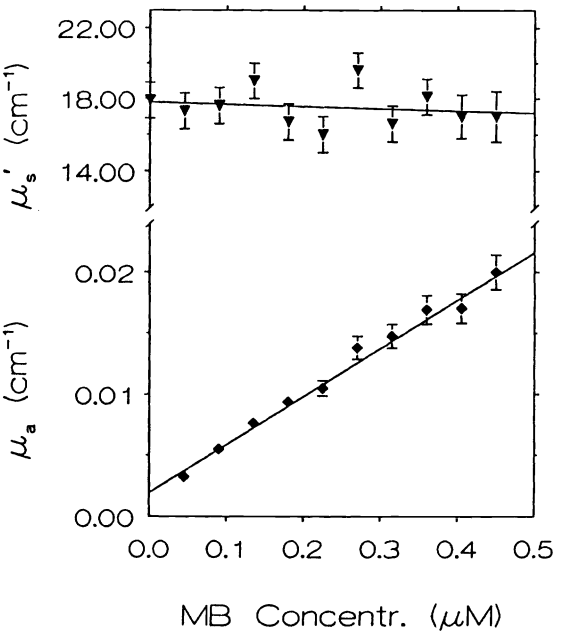

Fig. 4. Dependence of the values of μ_a and μ_s' at 680 nm on methylene blue concentration. The straight lines are obtained by a weighted least square method. When the error bars are not displayed they are on the order of the symbol dimensions.

4. DISCUSSION

The range of values for μ_a and μ_s' that can be measured with the frequency-domain LED method is determined by the following conditions:

i) in order for the diffusion model to be valid, μ_s' has to be much greater than μ_a (let's say $\mu_s' > 20\mu_a$);
ii) since the error on φ is on the order of tenths of a degree, we want φ to be at least 1°;
iii) an upper limit to μ_a and μ_s' is given by the amount of light signal at the greater distance (r_2). We assumed that the DC and AC signals at r_2 are not smaller than 1/300 times the signals at r_1.

By imposing these conditions we can evaluate the ranges of measurable values of μ_a and μ_s' using our instrument. The result is shown on a $\mu_s' - \mu_a$ log-log diagram in Fig. 5, where one can see that the typical

values of μ_a and μ_s' in tissues fall inside the measurable region of the $\mu_s' - \mu_a$ plane. The sensitivity of this technique to changes in the optical parameters can be evaluated by the relative errors on μ_a and μ_s'. These errors are typically within a few percent.

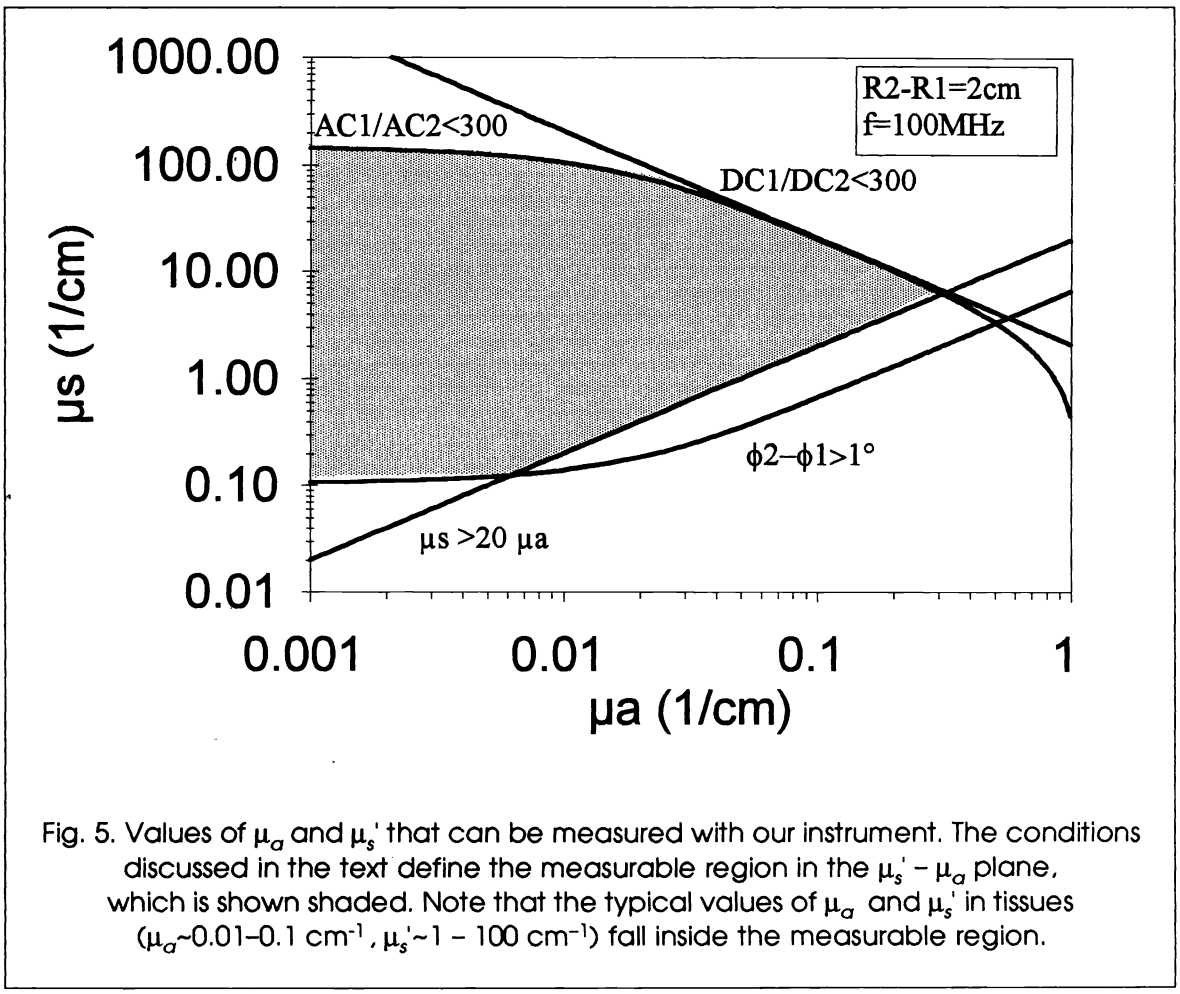

Fig. 5. Values of μ_a and μ_s' that can be measured with our instrument. The conditions discussed in the text define the measurable region in the $\mu_s' - \mu_a$ plane, which is shown shaded. Note that the typical values of μ_a and μ_s' in tissues (μ_a~0.01–0.1 cm^{-1}, μ_s'~1 – 100 cm^{-1}) fall inside the measurable region.

This work has dealt with a macroscopically homogeneous strongly scattering medium in a quasi-infinite geometry. Of course, the application of this technique to *in vivo* spectroscopy of tissues requires some further developments. First, biological tissues are not as homogeneous as an aqueous Liposyn solution. Second, they do not provide a quasi-infinite geometry (at least for non-invasive applications). The first point has already been considered,[9,19] and the reasonable results obtained *in vivo* on the basis of homogeneous medium models are encouraging. The second point requires the use of different boundary conditions. A first approach might be that of a semi-infinite geometry with source and detector placed on the surface. Although the diffusion approximation is not valid in such a geometry, it still constitutes a reasonable starting point.[9] We are currently working on this subject.

Several characteristics of the LED technique make it attractive from a practical standpoint:

- the instrumentation required by the LED technique can be made compact and portable;
- frequency-domain methods allow for fast acquisition times (~10 ms) which can provide real time measurements. Monitoring of fast dynamic processes is then feasible;

- the total power emitted by LEDs is typically less than a few mW and is distributed over a wide solid angle. This properties make LEDs safe for medical applications;
- the LED method is cost effective, especially when compared to other spectroscopic techniques.

5. ACKNOWLEDGMENTS

This work was performed at the Laboratory for Fluorescence Dynamics at the University of Illinois at Urbana-Champaign (UIUC), which is supported by the National Institutes of Health (NIH), grant RR03155 and by UIUC. This research is also supported by grant CA57032 from the NIH. The authors thank Julie Butzow for help in preparing this paper.

6. REFERENCES

1. G. Wagnieres, C. Depeursinge, P. Monnier, M. Savary, P. Cornaz, A. Chatelain, and H. van den Bergh, "Photodetection of Early Cancer by Laser Induced Fluorescence of a Tumor-Selective Dye: Apparatus Design and Realization," Proc. SPIE - Int. Soc. Opt. Eng. (USA) **1203**, 43-52 (1990).
2. M. S. Patterson, B. C. Wilson, J. W. Feather, D. M. Burns, and W. Pushka, "The Measurement of Dihematopotphyrin Ether Concentration in Tissue by Reflectance Spectrophotometry," Photochem. Photobiol. **46**, 337-343 (1987).
3. M. Cope and D. T. Delpy, "System for Long-Term Measurement of Cerebral Blood and Tissue Oxygenation on Newborn Infants by Near Infrared Transillumination," Med. Biol. Eng. Comput. **26**, 289-294 (1988).
4. B. C. Wilson and M. S. Patterson, "The Physics of Photodynamic Therapy," Phys. Med. Biol. **31**, 327-360 (1986).
5. S. L. Jacques and S. A. Prahl, "Modeling Optical and Thermal Distribution in Tissue During Laser Irradiation," Laser Surg. Med. **6**, 494-503 (1987).
6. F. F. Jobsis, J. H. Keizer, J. C. LaManna, and M. Rosenthal, "Reflectance Spectrophotometry of cytochrome aa_3 *in vivo*," J. Appl. Physiol. **43,** 858-872 (1977).
7. P. Parsa, S. L. Jacques, and N. S. Nishioka, "Optical Properties of Rat Liver Between 350 and 2200 nm," Appl. Opt. **28**, 2325-2330 (1989).
8. T. J. Farrel, M. S. Patterson, and B. Wilson, "A Diffusion Theory Model of Spatially Resolved, Steady-State Diffuse Reflectance for the Noninvasive Determination of Tissue Optical Properties *in vivo*," Med. Phys. **19**, 879-888 (1992).
9. M. S. Patterson, B. Chance, and B. C. Wilson, "Time Resolved Reflectance and Transmittance for the Noninvasive Measurement of Tissue Optical Properties," Appl. Opt. **28**, 2331-2336 (1989).
10. M. S. Patterson, J. D. Moulton, B. C. Wilson, K. W. Berndt, and J. R. Lakowicz, "Frequency-Domain Reflectance for the Determination of the Scattering and Absorption Properties of Tissue," Appl. Opt. **30**, 4474-4476 (1991).
11. J. B. Fishkin, E. Gratton, M. J. van de Ven, and W. W. Mantulin, "Diffusion of Intensity Modulated Near-IR Light in Turbid Media," in *Time Resolved Spectroscopy and Imaging of Tissues*, B. Chance editor, Proc. SPIE **1431**, 122-135 (1991).
12. J. B. Fishkin and E. Gratton, "Propagation of Photon-Density Waves in Strongly Scattering Media Containing an Absorbing Semi-Infinite Plane Bounded by a Straight Edge," J. Opt. Soc. Am. A **10**, 127-140 (1993).

13. W. F. Cheong, S. A. Prahl, and A. J. Welch, "A Review of the Optical Properties of Biological Tissues," IEEE J. Quantum Electron. **26**, 2166-2185 (1990).
14. Aldrich Chemical Company Laboratories, Milwaukee, Wisconsin (1993).
15. R. D. Spencer and G. Weber, "Measurement of Subnanosecond Fluorescence Lifetime with a Cross-Correlation Phase Fluorometer," Ann. N. Y. Acad. Sci. **158**, 361-376 (1969).
16. B. A. Feddersen, D. W. Piston, and E. Gratton, "Digital Parallel Acquisition in Frequency Domain Fluorometry," Rev. Sci. Instrum. **60**, 2929-2936 (1989).
17. G. M. Hale and M. R. Querry, "Optical Constants of Water in the 200 nm to 200 m Wavelength Region," Appl. Opt. **12**, 555-563 (1973).
18. H. J. van Staveren, C. J. M. Moes, J. van Marle, S. A. Prahl, and M. J. C. van Gemert, "Light Scattering in Intralipid-10% in the Wavelength Range of 400-1100 nm," Appl. Opt. **30**, 4507-4514 (1991).
19. E. M. Sevick, B. Chance, J. Leigh, S. Nioka, and M. Maris, "Quantitation of Time- and Frequency-Resolved Optical Spectra for the Determination of Tisuue Oxigenation," Anal. Biochem. **195**, 330-351 (1991).