Near infrared time-resolved spectroscopy in scattering and biological media

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Introduction

The ability to measure optical properties is absolutely necessary for many biomedical applications and various methods have been suggested to measure the absorption coefficient (μ_a), the scattering coefficient (μ_s) and the anysotropy g-factor. One of the most flexible techniques is the time-resolved spectroscopy in the diffuse reflectance (time-resolved reflectance: TRR) and transmittance (time-resolved transmittance: TRT) geometry. Generally TRR has been largely employed showing its advantages and limits. However, TRT can be useful in overcoming some of TRR's difficulties (for example, the maximum depth that can be probed, the influence of superficial effects and so on). Hence, in this paper, identical samples have been investigated with both TRR and TRT techniques. The experimental data have been compared with an analytical solution to the diffusion equation which pays particular attention to the boundary condition approximation and a comparison between the two different techniques has been attempted.

Theoretical description of time-resolved transmittance and reflectance

The transport of light in scattering media is usually described by diffusion approximation when adequate conditions have been respected. The diffusion equation is given by:

$$\frac{1}{c}\frac{\partial}{\partial t}\phi(\vec{r},t) - D\nabla^2\phi(\vec{r},t) + \mu_a\phi(\vec{r},t)\,S(\vec{r},t) \tag{1}$$

where $\phi(\vec{r},t)$ is the photon flux, D is the diffusion coefficient and $S(\vec{r},t)$ is the source term (usually assumed to be a Δ-function). Recently, analytical solutions taking into account the refractive index mismatch between the turbid medium and the surrounding medium have been proposed.[1] Previous results have shown that the effect of the refractive index mismatch cannot be disregarded especially for the transmittance and so the following expressions will be used in the analysis of our data.

$$T(d,t) = (4\pi Dc)^{-1/2}(t-t_0)^{-3/2}\exp[-\mu_a c(t-t_0)]\times$$
$$\times\sum_{m=\infty}^{+\infty}\left\{z_{1,m}\exp[-\frac{(z_{1,m})^2}{4Dc(t-t_0)}] - z_{2,m}\exp[-\frac{z_{2,m}{}^2}{4Dc(t-t_0)}]\right\} \tag{2}$$

where $\Delta = 1/(3m's)$, and
$z_1,m = d(1–2\ m)–4\ m\ z_e$,
$z2,m = d(1–2\ m)–(4\ m–2)z_e + z_0$,
$ze = 2AD$,
$A = 504.332880 – 2641.00214\ n + 5923.699064\ n^2 – 7376.355814\ n^3 + 5507.53041\ n^4 +$
$–2463.357945\ n^5 + 610.956547\ n^6, – 64.8047\ n^7$
and n represents the relative refractive index, i.e., the ratio between the refractive index of diffusing medium and that of the external medium.

$$R(\rho,d,t) = \frac{1}{2}(4\pi Dc)^{-3/2}(t-t_0)^{-5/2}\exp[-\mu_a c(t-t_0) - \frac{\rho^2}{4D(t-t_0)}] \times$$
$$\times \sum_{m=-\infty}^{+\infty}\left\{z_{3,m}\exp[-\frac{(z_{3,m})^2}{4Dc(t-t_0)}] - z_{4,m}\exp[\frac{z_{4,m})^2}{4Dc(t-t_0)}]\right\} \quad (3)$$

where $z_{3,m} = -2\ m\ d – 4\ m\ z_e – z_0$, $z_{4,m} = -2\ m\ d – (4\ m – 2)\ z_e + z_0$.

In our analysis we consider, in Equations. 2 and 3, n = 1.33 (nslab / next = 1.33/1 as we consider the refractive index of water). Following Contini *et al*.,[1] five dipoles should be retained in Equations 2 and 3 for maintaining the truncation error within 1%. To reduce it, we considered seven dipoles (m = 0, ± 1, ± 2, ± 3).

Experimental methods and materials

To investigate the time-resolved transmittance and reflectance techniques the experimental set-up previously described[2] was employed. This apparatus was mainly composed of a Ti:Sa laser (Coherent MIRA 900Dual) with a repetition rate of 76 MHz, a pulse duration of 130 fs and a maximum average power of 1.5 W at 800 nm, pumped by an Argon ion laser (Coherent SABRE 400). The detector was a streak camera (Hamamatsu C5680 with S1-IR extended photocatode) with a temporal resolution of a few picosecond in synchroscan mode. The investigation was usually performed at 800 nm. A small part of laser pulse was sent to a photodiode (Hamamatsu C1888) to obtain the trigger signal for the streak camera synchroscan sweep. Both in TRT and TRR measurements, curves of I vs t were obtained from a bidimensional CCD response. An interval of about 3 ns was taken into account. The analysis of these curves was carried on using a fitting algorithm both in TRT and TRR. A detailed description of the analysis method is reported elsewhere.[3]

Various intralipid solutions with distilled water and different scattering properties were investigated as their optical properties can be theoretically predicted and, consequently, they are among the best candidates in measurements aimed to test different experimental techniques. In particular, acqueous solution of Intralipid 10% (Pharmacia) at different volume concentrations (C) varying from 10%, 50% and up to the original concentration (say 100%) were investigated. The theoretical evaluation of μ'_s was obtained by Mie theory as in Reference 2. Particular care was placed in the choice of cuvette size to be sure that all the assumptions on which the diffusion approximation is based were also always largely satisfiedwith light concentrations. Moreover, in all cases the cuvette size and quality (optical glass) avoided boundary reflection problems.

Results and discussion

For the sake of brevity only experimental data on selected intralipid solutions have been here reported. In particular, experimental results obtained in transmittance geometry have been shown in Table 1.

Table 1. Experimental results concerning optical properties of intralipid solutions from TRT data.

C (%)	**d** (cm)	μ'_s **(theo)** (cm^{-1})	μ'_s **(exp)** (cm^{-1})	μ_a **(exp)** (cm^{-1})
10	2	7	7.0 ± 0.5	0.093 ± 0.005
50	1	34.9	39.3 ± 0.6	0.069 ± 0.002
100	1	70	71.0 ± 0.6	0.036 ± 0.003

Table 2. Experimental data concerning optical properties of intralipid solutions from TRR data

C (%)	μ'_s **(theo)** (cm^{-1})	μ'_s **(exp)** (cm^{-1})	μ_a **(exp)** (cm^{-1})
10	7	9.0 ± 0.3	0.0704 ± 0.0012
50	34.9	39.9 ± 1.6	0.036 ± 0.002
100	70	71.2 ± 2.0	0.029 ± 0.003

In Table 2 the experimental results from TRR measurements are reported for the same intralipid solutions. It's important to say that measurements have been performed at different distances between source and detector, but for this preliminary comparison a mean value for each solution is shown

In this initial stage of comparison between time-resolved TRR and TRT techniques both of them seem to be able to give experimental values of μ'_s in good agreement with theoretical prediction for the low absorbing solutions investigated here. In addition, TRR seems to be less sensitive to small variation in the scattering solutions (giving a small variation of μ'_s) and much more care has to be taken to avoid spurious signals. As far as μ_a is concerned no similar statements can be made because for these solutions only the value for water can be considered (μ_a equal to $5\ 10^{-2}$ cm^{-1}) as a reference value. As is evident much more experimental investigation on different experimental approaches in time-resolved spectroscopy has to be performed in order to assess the definite capabilities of time-resoved techniques to give good experimental values for optical properties of thick scattering samples. However, as can be seen from literature, some considerations can be made. In fact, in spite of the large use of these techniques in very complicated experimental situations with not trivial samples as biological ones, very few papers report systematic investigations on simple and reproducible samples. Time-resolved reflectance in a long term regime is surely the most used time-resolved method thanks to the fact that, in the $t \to +\infty$ limit, m_a can be determined directly by considering the asymptotic slope of $\log_e[R(\rho, d,t)]$ vs t. A problem with this method is that the more noisy part of the $R(\rho,d,t)$ v. t curve has to be used. In general, the fitting procedure has to be done on a large part of the experimental curve and μ'_s and μ_a values can be obtained. For a systematic study of this procedure using a photon counting system as the detector one can refer to Reference 4 in which intralipid solutions with and without ink are mainly considered. In this investigation some limits of TRR measurements have been indicated. One of these, regarding the accuracy of fitting when the experimental curves gets nearer to the system response curve, can be overcome using a streak camera detector. As far as TRT is concerned, streak camera measurements on well-characterised and reproducible samples have shown that μ'_s can be measured with good accuracy.[3]

Conclusions

A preliminary comparison between time-resolved transmittance and reflectance techniques has been performed. A systematic study,[3] using time-resolved TRT on simple samples (latex and intralipid solutions), seems to suggest that TRT is able to evaluate with good accuracy the scattering coefficients. Conversely, time-resolved TRR seems to be very useful in determining absorption coefficients. So the joint use of transmittance and reflectance measurement could aid in characterising optical properties of scattering media and in separating the contributions of the scattering coefficient from the anisotropy g-factor.

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References

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