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Near-Infrared Absorption and Scattering Spectra of Tissues *in Vivo*

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ABSTRACT

Tissue inhomogeneity is often neglected in the analysis of the spectral data collected in tissues. We have measured the absorption and reduced scattering spectra of the human forearm in the wavelength range 633-841 nm, and in the range of source-detector separations 0.7-3.3 cm. We found that the layered tissue structure due to the skin and adipose layers may have a significant effect not only on the data collected at different distances, but also on the data at 633 nm, where the optical absorbance is significantly higher than at the longer wavelengths considered. We conclude that the dependence of the optical penetration depth on the absorption and reduced scattering coefficients should be carefully considered in the quantitative analysis of the optical spectra of tissues.

Keywords: tissue spectroscopy, tissue inhomogeneity, hemoglobin saturation, arm ischemia.

1. INTRODUCTION

Near-infrared tissue spectroscopy is a non-invasive tool sensitive to various physiologically relevant parameters. Among these parameters, the hemoglobin concentration and saturation are of particular interest because the hemoglobin is the dominant near-infrared chromophore in tissues. In the past few years, time-resolved techniques have been introduced to perform quantitative tissue spectroscopy,[1] aiming at the determination of absolute values of the hemoglobin concentration and saturation in tissues.[2-4] These quantitative approaches use a physical model (typically diffusion theory) for light propagation in tissues, and some assumptions about the boundary conditions and the spatial distribution of the optical properties. In particular, it is often assumed that tissues are macroscopically homogeneous, which is obviously only a first order approximation. Several studies have recently considered a two-layered structure as a better approximation of tissue inhomogeneity.[5-8] The influence of the skin and adipose tissue layers have been investigated both by Monte Carlo simulations[9] and by *in vivo* measurements.[10,11]

We have measured the absorption and reduced scattering spectra of the human calf muscle at different source-detector separations in order to investigate the influence of tissue inhomogeneity on the quantitation of hemoglobin concentration and saturation. Specifically, we have investigated the effect of the layered tissue structure, which results in a depth dependence of the optical properties.

2. METHODS

We have used a modified version of a frequency-domain tissue oximeter (Mod. No. 96208, ISS, Inc., Champaign, IL) which affords measurements at eight discrete wavelengths (633, 670, 751, 776, 786, 814, 830, and 841 nm) and two parallel acquisition channels.[12] The eight laser diodes are intensity modulated at a frequency of 110 MHz, they are electronically multiplexed at a rate of 50 Hz (each light source is on for 20 ms), and they are coupled to 400 μm-core-diameter optical fibers. These eight source fibers are assembled in a fiber bundle of rectangular section having an internal size of 2.4×1.2 mm^2. This eight-wavelength fiber bundle is mechanically scanned on the forearm muscle over a source-detector distance range of 0.7-3.3 cm (see Fig. 1). The scanning step is 0.1 cm, and the acquisition time per step is set to 3 s. The detector fibers, a fiber bundle 1 mm in diameter for channel A and a fiber bundle 3 mm in diameter for channel B, are placed in contact with the forearm. By contrast, the eight-fiber source bundle is kept about 1 mm above the skin to avoid any contact that could cause a variable fiber-to-skin optical coupling during the linear scan. The experimentally determined intensity and

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phase slopes versus source-detector distance are used to calculate the absorption and the reduced scattering coefficients at each wavelength.[13]

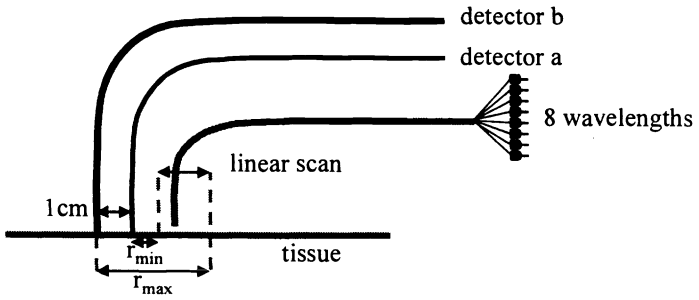


Fig.1. Geometrical arrangement of the multi-distance measurement on the forearm. r_{min} = 0.7 cm, r_{max} = 3.3 cm.

The experimental absorption coefficients at the eight wavelengths have been corrected by an assumed water concentration in tissue of 70%. This means that the water-corrected $\mu_a(\lambda)$ has been written as:

$$\mu_a(\lambda) = \mu_a^{exp}(\lambda) - 0.7\mu_a^{water}(\lambda) \ , \tag{1}$$

where $\mu_a^{exp}(\lambda)$ is the measured tissue absorption coefficient, and $\mu_a^{water}(\lambda)$ is the absorption coefficient of water. The water-corrected absorption spectrum has been fitted with a linear combination of the absorption spectra of oxy- and deoxy-hemoglobin:

$$\mu_a^{fit}(\lambda) = \varepsilon_{HbO2}(\lambda)[HbO_2] + \varepsilon_{Hb}(\lambda)[Hb] \ , \tag{2}$$

where the concentrations of oxy-hemoglobin ($[HbO_2]$) and deoxy-hemoglobin ([Hb]) were the fitted parameters. For the extinction coefficients of oxy-hemoglobin (ε_{HbO2}) and deoxy-hemoglobin (ε_{Hb}), we have used the values reported by Wray *et al.*[14] The minimization of the sum of the squares of the residuals (i.e. $\sum_i[\mu_a^{fit}(\lambda_i) - \mu_a(\lambda_i)]^2$) leads to a linear system whose solution gives the following best fit concentrations of oxy- and deoxy-hemoglobin:

$$[HbO_2] = \frac{\left(\sum_i \mu_a(\lambda_i)\varepsilon_{HbO2}(\lambda_i)\right)\left(\sum_i \varepsilon_{Hb}^2(\lambda_i)\right) - \left(\sum_i \mu_a(\lambda_i)\varepsilon_{Hb}(\lambda_i)\right)\left(\sum_i \varepsilon_{HbO2}(\lambda_i)\varepsilon_{Hb}(\lambda_i)\right)}{\Delta} \ , \tag{3}$$

$$[Hb] = \frac{\left(\sum_i \mu_a(\lambda_i)\varepsilon_{Hb}(\lambda_i)\right)\left(\sum_i \varepsilon_{HbO2}^2(\lambda_i)\right) - \left(\sum_i \mu_a(\lambda_i)\varepsilon_{HbO2}(\lambda_i)\right)\left(\sum_i \varepsilon_{HbO2}(\lambda_i)\varepsilon_{Hb}(\lambda_i)\right)}{\Delta} \ , \tag{4}$$

where $\Delta = \left(\sum_i \varepsilon_{HbO2}^2(\lambda_i)\right)\left(\sum_i \varepsilon_{Hb}^2(\lambda_i)\right) - \left(\sum_i \varepsilon_{HbO2}(\lambda_i)\varepsilon_{Hb}(\lambda_i)\right)^2$, and i is the wavelength index which numbers the various wavelengths used.

Our objective is to investigate the effect on quantitative tissue spectroscopy of the variability of the tissue optical properties with depth. Such a variability in the forearm is associated with the presence of the skin and adipose tissue layers above the skeletal muscle. We have examined three healthy volunteers having an adipose tissue layer of different thickness. The relevant characteristics of the three subjects are listed in Table I.

Table I. The three subjects examined.

Subject #	Sex	Age (y)	Adipose tissue thickness at the forearm (cm)
1	F	26	0.3
2	M	35	0.6
3	F	29	1.1

3. RESULTS

Figure 2 shows the absorption and the reduced scattering spectra measured on the forearm of the three subjects. Panels (a), (b), and (c) refer to subjects 1, 2, and 3, respectively. The symbols in the spectra of Fig. 2 are the experimental data points (after water correction in the case of the absorption coefficient). The lines in the scattering spectra are linear fits shown as an aid to the eye in evaluating the spectral dependence of the scattering. The lines in the absorption spectra are the best fits of a linear combination of the oxy- and deoxy-hemoglobin (as described in Section 2) to the seven points in the wavelength range 670-841 nm. We have excluded the first point (at 633 nm) in the evaluation of the fit, because its inclusion caused a significantly worse fit. This result indicates that the absorption coefficient at 633 nm is consistently underestimated. We have found this result also in other measurements, not reported here, in additional subjects, on both forearm and calf muscles. We will return to this point in the Discussion section. In Fig. 2, we report the spectra measured using three different ranges of source-detector distance. The larger the distance, the larger the optical penetration depth into the tissue. Figure 3 shows the total hemoglobin concentration and the hemoglobin saturation recovered in the three subjects from the fit at each of the three ranges of distance considered.

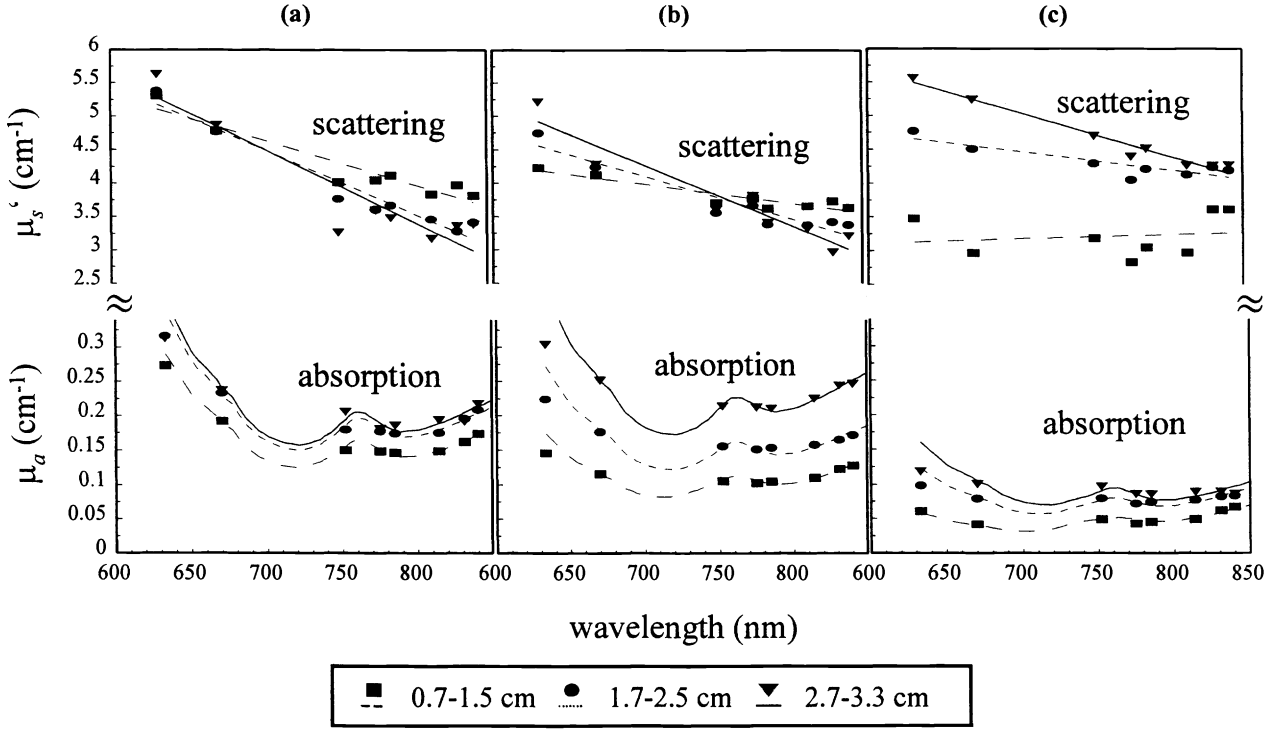


Fig. 2. Absorption and reduced scattering spectra measured at rest on (a): subject #1, (b): subject #2, and (c): subject #3. The experimental points refer to three different ranges of source-detector separations: 0.7-1.5 cm (squares), 1.7-2.5 cm (circles), and 2.7-3.3 cm (triangles). The absorption spectra (with the exclusion of the point at 633 nm) are fitted with a linear combination of the absorption of oxy- and deoxy-hemoglobin. The scattering spectra are fitted with a straight line to show the spectral dependence. The hemoglobin concentration and saturation obtained from the fit of the absorption spectra are reported in Fig. 3.

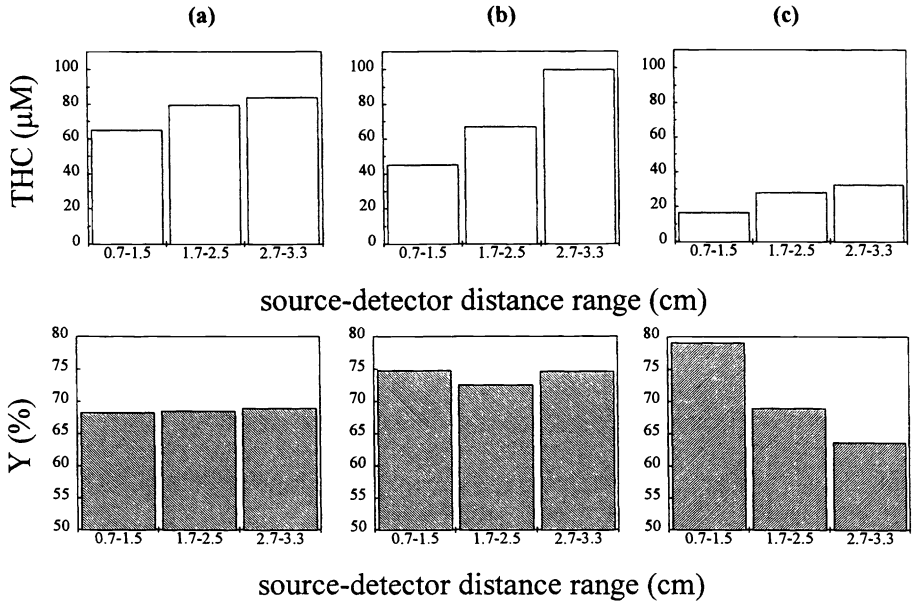
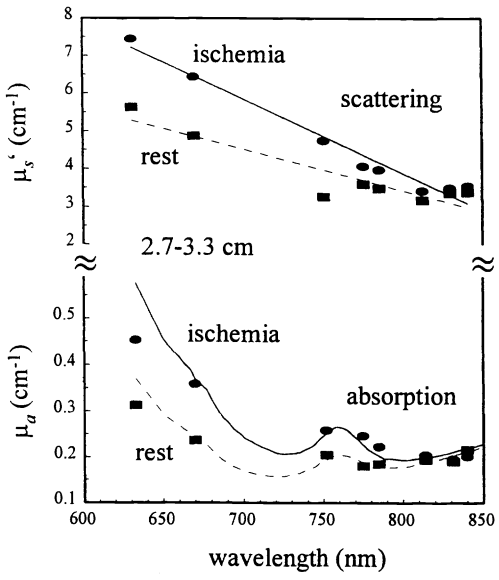


Fig. 3. Total hemoglobin concentration and hemoglobin saturation obtained from the fit of the absorption spectra of Fig. 2 at the three different ranges of distance considered. (a): subject #1, (b): subject #2, (c): subject #3.

We have measured the optical spectra of the forearm during a pneumatic-cuff-induced ischemia. Figure 4 shows the absorption and reduced scattering spectra measured on subject #1 at rest and after 3 min of arterial occlusion at the upper arm (by a pneumatic cuff inflated at 220 mmHg). We verified that after 3 minutes of ischemia, we reached a situation where the tissue optical properties were approximately constant with time. Consequently, the total acquisition time for the spectral, multi-distance data (about 50 s) was not a limiting factor in this case. The range of distances considered in Fig. 3 is the largest of the three, i.e. 2.7-3.3 cm. The values of hemoglobin concentration and saturation before and during ischemia are reported in the Table next to Fig. 4. We note the small increase in the hemoglobin concentration and the significant drop in the hemoglobin saturation caused by ischemia. We also note that the underestimation of the absorption at 633 nm is more significant during ischemia than at rest.



	rest	isch.	
THC	84	92	μM
Y	69	50	%

Fig. 4. Absorption and reduced scattering spectra measured at rest (squares) and 3 minutes after ischemia (circles) on subject #1. The experimental absorption spectra are fitted with a linear combination of the absorption spectra of oxy- and deoxy-hemoglobin. The total hemoglobin concentration (THC) and the hemoglobin saturation (Y) obtained from the fits are reported in the above Table. The experimental scattering spectra are fitted with a straight line to show the spectral dependence.

4. DISCUSSION

The key to discuss and interpret our data is the dependence of the optical penetration depth on the source-detector separation, and on the tissue optical properties. Patterson *et al.* have determined an empirical relationship for the average photon penetration depth in terms of the source-detector separation r, and the absorption (μ_a) and reduced scattering (μ_s') coefficients.[15] Such a relationship predicts a dependence of the penetration depth on $r^{1/2}$ and on $(\mu_a\ \mu_s')^{-1/4}$. Therefore, the optical penetration depth, as one would predict intuitively, increases with r and decreases with μ_a and μ_s'.

The absorption spectra at three different ranges of source-detector separation (Fig. 2) show the relevance of the superficial adipose tissue layer in near-infrared tissue spectroscopy. In subject #1 (adipose tissue thickness of 0.3 cm), the two upper spectra, being almost coincident, are likely representative of the underlying skeletal muscle. In subject #3 (adipose tissue thickness of 1.1 cm), the low absorption values in all three spectra suggest that we are mostly probing the adipose layer even at the largest source-detector distance employed. Subject #2 (adipose tissue thickness of 0.6 cm) is an intermediate case, where increasing the source-detector separation determines a transition between a case where we mostly probe the adipose layer (low absorption), to a case where we mostly probe the skeletal muscle (high absorption). This finding is in agreement with our previously reported results on two-layered tissue-like phantoms.[8]

We attribute the underestimation of μ_a at 633 nm to a reduced optical penetration depth caused by the higher absorption and reduced scattering coefficients at that wavelength compared to longer wavelengths. The lower value of μ_a at a shallower depth is confirmed by the measurements at shorter values of r (see Fig. 2). We further observe that Fig. 2 shows that the spectral dependence of the reduced scattering coefficient is less pronounced at shallower depths (adipose layer) than deeper in the tissue (skeletal muscle). In Fig. 4, the larger underestimation of μ_a at 633 nm during ischemia with respect to rest is due to the larger value of μ_a (and also of μ_s') which further reduces the optical penetration depth. We also note the change in the spectral dependence of the reduced scattering coefficient caused by ischemia (see Fig. 4).

5. CONCLUSION

We conclude by pointing out that the tissue inhomogeneity may not only affect the data taken at different source-detector separations. Tissue inhomogeneity must also be taken into account in the analysis of spectral data, if the optical coefficients vary significantly in the spectral range considered. Our results indicate that tissue spectroscopy over a broad spectral range, to be quantitative, requires a means for depth discrimination. This can be achieved, to a first order, using the solution to the diffusion equation for two-layered media.[6]

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