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Assessment of Muscle Optical Properties by Double Distance TD NIRS Measurements: a Simulation and In-Vivo Study

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Abstract: We propose an analysis method for TD NIRS based on measurements at two source-detector distances specific for a two-layer geometry. The method was verified by numerical simulations and applied in-vivo on a muscle study. © 2025 The Author(s)

1. Introduction

The use of near infrared spectroscopy (NIRS) and of time domain (TD) NIRS on muscle for the evaluation of the oxidative metabolism is growing [1]. Typically, the muscle tissue is beneath other tissues, such as dermis and fat. For this reason, in its simpler approximation, a two-layer structure is considered to study muscles. Even if different approaches have been considered for retrieving the optical properties of two-layer media [2, 3], this remains a big challenge for analysis methods, also considering the information one can acquire (*e.g.* thickness of the upper layer, multi-distance measurements) and the variability of optical parameters to reconstruct. To this extent, we can note that for very small values of the superficial adipose tissue thickness (ATT) a homogeneous structure can be assumed. However, when ATT increases, results obtained with a homogeneous model became less reliable and more sophisticated analysis methods have to be considered. In this work, we propose an inversion method for TD NIRS data, based on measurements performed at two source-detector distances, that focus on the optical properties of the lower layer. This method was tested on numerical simulations and applied to an *in-vivo* study involving measures on the vastus lateralis muscle of old subjects that underwent femur fracture.

2. Material and methods

2.1 Simulations

In order to assess the sensitivity of the retrieved optical properties to the lower layer in a two-layer structure, we performed numerical simulations considering the solution of the diffusion equation for this kind of structure. In particular, we considered a two-layer geometry, with fixed optical properties of the upper layer (absorption coefficient μ_{a1} = 0.05 cm^{-1} and reduced scattering coefficient μ'_{s1} = 10 cm^{-1}) and variable layer thickness (*s* from 0.25 to 1.5 cm) and optical properties of the lower layer (μ_{a2} from 0.015 to 0.6 cm^{-1}, and μ'_{s2} from 3.5 to 12.5 cm^{-1}). Measures at two source-detector distances were simulated (ρ = 1.5 and 3 cm) in a reflectance geometry. Finally, the simulated data were convolved with a realistic instrument response function. For the inversion process, a nonlinear fitting procedure based on the Levenberg-Marquardt algorithm was used, considering different possible ways to exploit the experimental data to obtain the absorption coefficient μ_{a2} of the lower layer:

- Method 1: fit the time-resolved curves measured at the longer ρ with a homogeneous model to obtain μ_{a2};
- Method 2: fit the time-resolved curves measured at the shorter ρ with a homogeneous model to fix μ_{a1} and μ'_{s1}. Then, fix $\mu'_{s2} = \mu'_{s1}$. Finally, fit the tail of the curves measured at the longer ρ with a two-layer model to retrieve μ_{a2}.
- Method 3: fit the time-resolved curves measured at the shorter ρ with a homogeneous model to fix μ'_{s1}. Then, fit the time-resolved curves measured at the longer ρ with a homogeneous model to fix μ'_{s2}. Finally, fit simultaneously the curves acquired at the two ρ with a two-layer model to retrieve both μ_{a1} and μ_{a2}.

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2.2 In-vivo

The *in-vivo* study was approved by the Ethical Committee of Istituto Ortopedico G. Pini and conducted in accordance with the Declaration of Helsinki. 19 old bedridden patients after surgery for a hip fracture (female, 79.0 ± 6.7 years) signed the informed consent before taking part in the study. The measurement time-point was set at 6-8 days after the femur fracture and around 3 days after the surgery. During the acquisition, subjects were seated on a custom chair that allowed to fix the ankle of the non-surgical leg in such a way as to have an angle of 120° and not to allow movement to the leg itself [4]. Subjects were asked to perform three isometric contractions with the maximum force they were able to exert. The force exerted was measured with a load cell inserted in the holder. Averaging the three trials, the maximum voluntary contraction (MVC) was determined. With an ultrasonographic (US) exam, the superficial ATT and the muscular fiber pennation angle (PA) was measured.

The TD NIRS acquisitions were performed with the medical device described in Re *et al.* [5], developed at the Department of Physics of Politecnico di Milano. The optical custom probe was placed on the distal vastus lateralis muscle of the non-surgical leg. Two source-detector distances were employed: $\rho = 1.5$ and 3 cm, allowing for two measurement points, aligned parallel to the muscular fibers. The subjects were required to rest for 60 s, while a baseline was acquired, with an acquisition rate of 1 Hz. We performed a signal quality check, based on the number of photons acquired and the fit procedure goodness. On this basis, no subjects were excluded from the study.

The absolute values of the optical properties of the vastus lateralis muscle were obtained with method 3 explained in Sec. 2.1, as it resulted as the most independent from ATT values (see Sec. 3.1). The absolute values for the hemodynamic parameters, i.e. oxy-, deoxy- and total- hemoglobin content (O_2Hb, HHb and tHb respectively, expressed in μM) and the tissue oxygen saturation (SO_2, expressed in %), were calculated with the Lambert-Beer Law. The baseline values of the previous parameters were calculated as the average of the last 10 seconds of the acquisition. Scatterplots and Pearson correlation coefficient were used to identify linear relationship between couple of variables. Principal component analysis (PCA) were also run to get an overview of correlations among the hemodynamic parameters and the personal (age) and anthropometric (MVC, ATT, PA) ones.

3. Results and discussions

3.1 Simulations

In Fig. 1, there are reported μ_{a2} and μ'_{s2} retrieved on simulated data when μ_{a2} is varied. The 3 inversion methods described in Sec. 2.1 are considered. From an inspection of this figure, it results that method 3 shows the better results in terms of independence of μ_{a2} from *s*. As for the reduced scattering coefficient, apart from low values of μ_{a2}, it is almost fixed to the value of the upper layer.

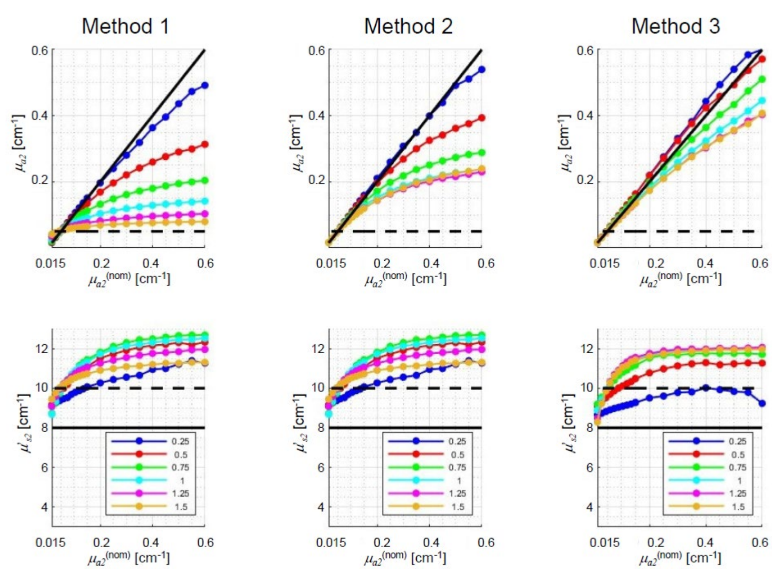

Fig 1. Absorption (first row) and reduced scattering (second row) coefficients of the lower layer as a function of the nominal absorption coefficient of the lower layer. Different colors represent different thicknesses (in cm) of the upper layer. Nominal optical parameters of the upper layer (dashed black lines) and lower layer (solid black lines) are also reported.

3.2 In-vivo

The group anthropometric data for our population were: ATT = 10.7 ± 3.8 mm, PA = 7.8 ± 4.3°, MVC = 289.6 ± 100 N. In Table 1 the group optical and hemodynamic characterization is presented.

Table 1. Group optical characterization for the vastus lateralis muscle, *i.e.* the lower layer of the two-layer structure. Average, median and standard deviation are shown for the absorption coefficient, μ_{a2}, and reduced scattering coefficient, μ'_{s2} measured at 688 nm (RED) and 828 nm (IR).

	μ_{a2} [cm^{-1}]		**μ'_{s2} [cm^{-1}]**			**O_2Hb [µM]**	**HHb [µM]**	**tHb [µM]**	**SO_2 [%]**
	RED	**IR**	**RED**	**IR**					
Average	0.23	0.19	8.84	8.10		46.1	25.8	71.9	64.7
Median	0.15	0.14	8.83	8.17		42.7	22.3	64.1	64.1
Standard Deviation	0.31	0.22	0.73	0.70		14.3	10.5	24.2	3.8

The correlations between the hemodynamic parameters and the ATT are negative and moderate ($-0.54 < r < -0.46$) except for SO_2, where the correlation is moderate but positive. A weak correlation was found between hemodynamics parameter and age ($r \approx 0.3$), while no correlation is shown for MVC and PA ($r < 0.07$). This analysis and the scatterplots confirm the tendence shown in the simulations: an underestimation of tHb and overestimation of SO_2 with the increase of ATT. Considering the PCA score plot for tHb, we can observe that the first two principal components (PCs), that explain more than 95% of the variation, are tHb and MVC, while the ATT has a weak correlation with these parameters (perpendicular vectors). For the PCA with SO_2, we have the AGE instead of MVC as PCs, which has a weak positive correlation with ATT. These results suggest that also if there is an error in the extrapolation of the hemodynamic parameters, due to the proposed method, this is not influencing negatively the final estimation, since there are other factors, such as MVC, which is related to muscular status, and age that are mostly influencing the variability of these parameters. This is true, in this kind of old and bedridden population, where there is a huge variability of conditions. It will be interesting, in the future, to investigate the same parameters in a more homogenous population such as young.

3. Conclusion

We have presented an analysis method for TD NIRS based on measurements performed at two source-detector distances specific for a two-layer geometry. This method is well suited for applications on muscle, in particular when the ATT is not negligible. We tested three different variants of this method, identifying the one which minimizes the errors in the retrieval of the optical properties of the lower layer. It was also successfully applied on a population of 19 female old bedridden patients.

References

[1] T. J. Barstow, "Understanding near infrared spectroscopy and its application to skeletal muscle research", J Appl Physiol **126**: 1360–1376 (2019).

[2] F. Martelli, S. Del Bianco, L. Spinelli, S. Cavalieri, P. Di Ninni, T. Binzoni, A. Jelzow, R. Macdonald, H. Wabnitz, "Optimal estimation reconstruction of the optical properties of a two-layered tissue phantom from time-resolved single-distance measurements," J. Biomed. Opt. **20**, 115001 (2015).

[3] R. Re, D. Contini, L. Zucchelli, A. Torricelli, and L. Spinelli, "Effect of a thin superficial layer on the estimate of hemodynamic changes in a two-layer medium by time domain NIRS," Biomed. Opt. Express **7**, 264-278 (2016).

[4] R. Re, A. Scano, O. Amata, L. Spinelli, A. Tomba, C. Brambilla, A. Frizziero, A. V. Caserta, R. Cubeddu, A. Torricelli and D. Contini, "A personalized clinical assessment: multi-sensor approach for understanding musculoskeletal health in the frail population", BioMed Eng OnLine **23**, 91 (2024).

[5] R. Re, I. Pirovano, D. Contini, L. Spinelli, and A. Torricelli, "Time Domain Near Infrared Spectroscopy Device for Monitoring Muscle Oxidative Metabolism: Custom Probe and In Vivo Applications," Sensors **18**, 1–13 (2018).