

# Frequency-Domain Optical Measurements *in Vitro* on Two- and Three-Layered Tissue-Like Phantoms, and *in Vivo* on Infant Heads

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## Abstract

We have performed frequency-domain optical measurements on the surface of two- and three-layered turbid media (*in vitro*) and on infant heads (*in vivo*). For the *in vitro* experiment we measured a number of combinations of one layer (thickness in the range 0.1-1.6 cm) on top of one semi-infinite block, with or without a clear layer (thickness of 0.1 cm) sandwiched in between. For the *in vivo* experiment, we did measurements on 20 preterm infants at the Meyer Children's Hospital in Florence, Italy. In the two-layered samples, we measured the effective absorption and reduced scattering coefficients with a multi-distance method based on data taken at source-detector distances in the range 1.5-4.5 cm. We found that the effective optical coefficients reproduce those of the underlying block if the superficial layer is less than about 0.4 cm thick, whereas they are representative of the superficial layer if it is more than about 1.3 cm thick. Larger source detector separations reduce the effect of the superficial layer on the recovery of the effective optical coefficients. Measurements in the presence of the clear layer gave results that did not reproduce, even qualitatively, the *in vivo* measurements on infant heads.

**Key Words:** (300.6500) Time resolved spectroscopy; (170.7050) Turbid media; (300.1030) Absorption; (170.0170) Medical optics and biotechnology.

## 1. Introduction

Near-infrared spectroscopy is a promising technique for the non-invasive study of biological tissues *in vivo*. To obtain quantitative information about the optical coefficients of tissues, one often makes the assumption of tissue homogeneity. This assumption can only be approximately

valid in tissues, and it appears to be violated in the non-invasive study of the brain, where the inhomogeneity due to the cerebrospinal fluid (CSF), skull, and scalp can significantly affect the optical measurements. For this reason, several studies have been devoted to the investigation of light propagation in layered strongly scattering media.<sup>1-3</sup> In this contribution, we consider frequency-domain experimental results obtained *in vitro* on two- and three-layered media, and *in vivo* on infant heads.

## 2. Materials and Methods

For the *in vitro* experiment, we have used gelatin to prepare 5 blocks (about 5 cm thick) and 21 layers (thickness ranging from 0.1 to 1.6 cm). Different amounts of white paint and black India ink were added to the gelatin samples to obtain media with different optical properties. One of the 21 layers does not contain any paint or ink and is optically transparent in the visible/near-infrared spectral region (clear layer). Fig. 1 reports the optical properties of the 5 blocks (measured with the frequency-domain multi-distance method) at 750 nm. The blocks are labeled with progressive numbers from 1 to 5. The layers have the same optical properties of the corresponding blocks. The letters a, b, c, d, are used to indicate different thicknesses (the thickness increases from a to d). For the two-layered media study, we measured all possible combinations (100) of one superficial layer on top of one underlying block. For the three-layered media study, we measured 30 combinations where the clear layer is sandwiched between one superficial layer and one underlying block (we used the 5 blocks and layers 1a, 1b, 1c, 4a, 4b, and 4c).

The *in vivo* measurements were performed on 20 preterm infants at the Meyer Children's Hospital in Florence, Italy. We acquired data in four different positions on the head (temple, forehead, anterior fontanelle, and posterior fontanelle) with the infants resting in their bassinets.

The data were acquired at 750 and 840 nm with a frequency-domain (110 MHz), tissue spectrometer<sup>4</sup> (ISS, Inc. Model 96208). In the *in vitro* measurements we acquired data at source-detector distances ( $r$ ) ranging from 1.5 to 4.5 cm. In the *in vivo* experiment we employed a flexible measuring probe (capable of fitting the shape of the infant heads) with source-detector distances ranging from 2.1 to 3.6 cm. The data were analyzed by using the solution to the diffusion equation for a semi-infinite, homogeneous medium. The purpose of this study is to investigate the effect of superficial layers on the performance of the frequency-domain multi-distance measurement protocol based on the equations for homogeneous media.

### 3. Results

**Two-layered media experiment.** When the thickness of the superficial layer is  $\leq 0.4$  cm, the multi-distance method correctly recovers the optical properties of the underlying block. In the case of the thickest layers (1.3-1.6 cm), the recovered values of  $\mu_a$  and  $\mu_s'$  are close to those of the upper layer. Figure 2 shows the results for a superficial layer of intermediate thickness (0.55 cm, layer 4c) on top of the five different blocks. In this case, the measurement at larger source-detector distances accurately reproduces the block properties, even in the presence of a 0.55 cm thick layer. In all cases, we have found that by considering larger source-detector distances (circles in the figure), we get a more accurate recovery of the optical coefficients of the underlying block. However, this improvement is not substantial in the range of distances considered by us.

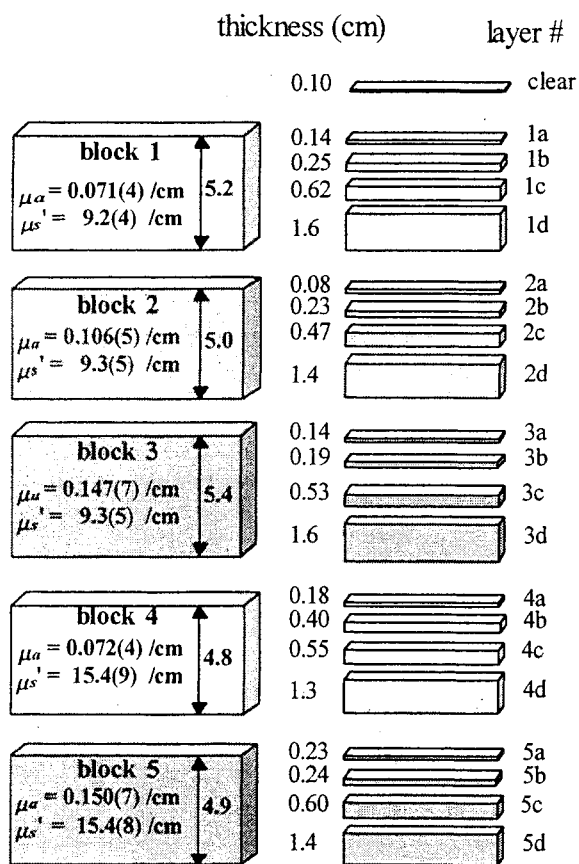


Fig. 1. The effectively semi-infinite blocks and the layers employed in our experimental study. The optical properties of each batch at 750 nm are indicated inside the blocks. The numbers in parenthesis give the error in the last digit of the corresponding parameter. The thickness of each block and layer is also reported in the figure. The clear layer is made of the same gelatin material, without adding any absorbing or scattering compound.

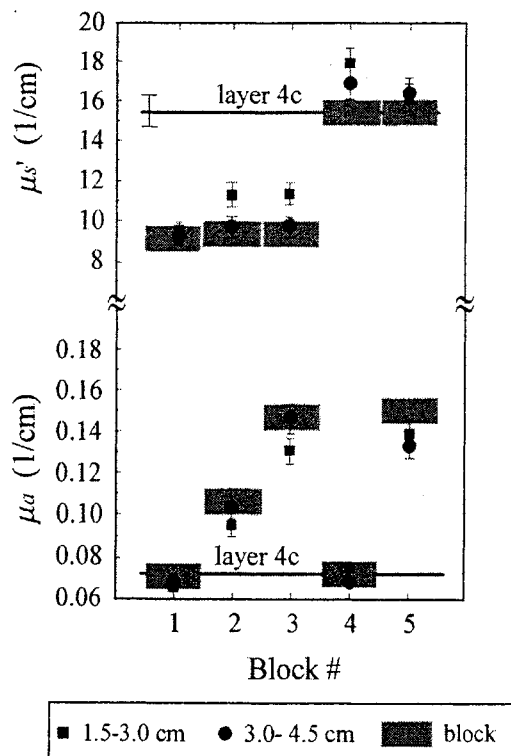


Fig. 2. Effective optical properties measured at 750 nm for the case of layer 4c (0.55 cm thick) on top at each of the five blocks. The squares are the measurements obtained with source-detector distances between 1.5 and 3.0 cm, while the circles are obtained from the range 3.0 to 4.5 cm. The thick horizontal bars represent the optical properties of the blocks. The thin horizontal lines indicate the optical properties of the layer.

To look at our measurements under a different perspective, we consider the  $r$ -dependence of the average value (dc) and phase of the modulated intensity. The symbols in Fig. 3 represent the experimental values of  $\ln(r^2dc)$  and phase, for the case of layer 1c on top of block 5. In this case, the difference in the optical properties of the layer and the block is the largest among our samples. Furthermore, the layer thickness (0.60 cm) has an intermediate value between the cases where the effective optical properties reproduce those of the block (thickness  $\leq 0.4$  cm) or those of the layer (thickness  $\geq 1.3$  cm). For comparison, we also show the corresponding straight lines obtained in the semi-infinite geometry on block 1 (thin lines) and block 5 (thick lines). Since, to a first approximation,  $\mu_a$  and  $\mu_s'$  can be calculated from the slopes of  $\ln(r^2dc)$  and phase vs.  $r$ , one can see that the presence of the superficial layer affects the recovered optical parameters (because it changes the slopes). Nevertheless, the experimental points are approximately aligned in the whole range of distances measured. Consequently, the multi-distance method can be applied even in the presence of a superficial layer. The effective absorption (reduced scattering) coefficient recovered in this layer/block configuration is  $\mu_a = 0.18 \text{ cm}^{-1}$  ( $\mu_s' = 10.1 \text{ cm}^{-1}$ ). These values deviate by 20% (-34%) from the coefficients of block 5, and by 154% (10%) from the coefficients of layer 1c.

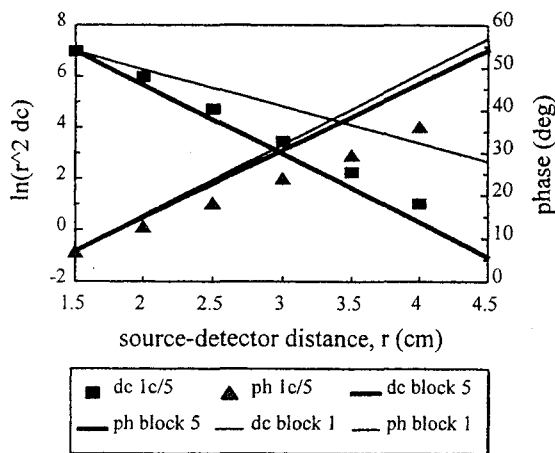


Fig. 3. Normalized experimental values of  $\ln(r^2dc)$  and phase versus source-detector distance for the various samples indicated in the legend.

**Three-layered media experiment.** This experiment was conducted on phantoms where a 0.1 cm-thick clear layer was sandwiched between one superficial layer and one underlying block. Since the two layers and the block were made out of the same material there was no index of refraction mismatch at the interfaces.

In Fig. 4 we show the comparison between the  $\ln(r^2dc)$  (panel (a)) and phase (panel (b)) measured in a three-

layered medium (layer 4c on top, clear layer in the middle, block 3 at the bottom), in a two-layered medium (layer 4c on top of block 3), and in blocks 3 and 4 alone. The results in all of the layer/block configurations show the large effect of the clear layer on the optical data. From the  $r$  dependencies of the dc and phase, we can see that there is a light channeling effect through the clear layer as observed by Okada *et al.*<sup>2</sup> The dc intensity decreases slowly with distance, and the phase tends to flatten. Figure 4(b) shows that the phase increases by only 2.7 deg/cm in the presence of the clear layer, while it increases by 13.5 deg/cm in the absence of the clear layer.

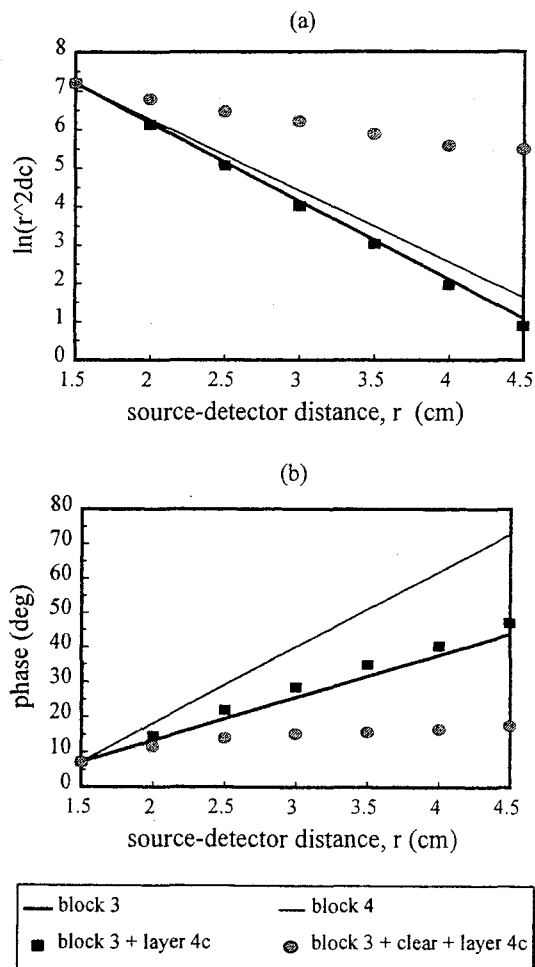


Fig. 4. Normalized experimental values of  $\ln(r^2dc)$  (panel (a)) and phase (panel (b)) for the different cases indicated in the legend.

**In vivo experiment.** Figure 5 shows the comparison between the  $\ln(r^2dc)$  and the phase measured in the previously considered three-layered medium (layer 4c on top, clear layer in the middle, block 3 at the bottom), and *in vivo* on the temple area of the head of a female infant (born on the 34<sup>th</sup> week of gestation; age: 12 days; weight: 1.9 kg;

head circumference: 30 cm). The propagation of light in infant heads does not seem to be affected by the channeling effect that we see in the phantom measurements in the presence of the clear layer. The average phase slope we measured in 20 infants in the temple area is  $12 \pm 2$  deg/cm. The average phase slope measured in the phantoms containing the clear layer (30 cases) is  $4 \pm 2$  deg/cm. The intensity (panel (a)) is also much more attenuated by increasing the source-detector distance in the infant head than in the *in vitro* case with the clear layer.

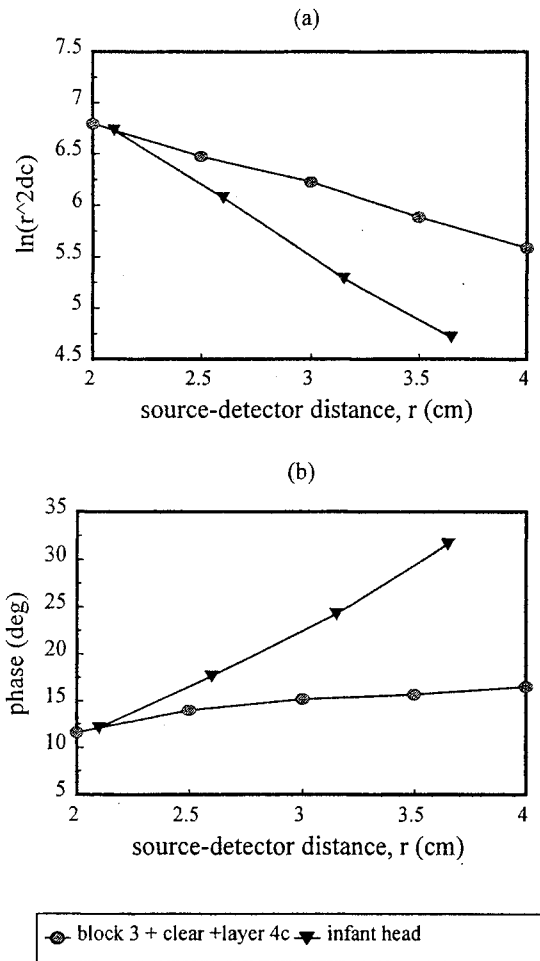


Fig. 5. Normalized experimental values of  $\ln(r^2dc)$  (panel (a)) and phase (panel (b)) in a phantom containing a clear layer and *in vivo*. The optical properties of the infant head recovered from these data are  $\mu_a = 0.087 \text{ cm}^{-1}$ , and  $\mu_s' = 6.0 \text{ cm}^{-1}$  at 750 nm.

#### 4. Discussion

For non-invasive optical studies of skeletal muscles, two-layered media constitute a model of the superficial skin/fat layer and the underlying muscle. Our study was based on the frequency-domain multi-distance method. The intensity

modulation frequency was 110 MHz, the range of source-detector separations was 1.5-4.5 cm, and the range of optical properties was  $0.071\text{-}0.15 \text{ cm}^{-1}$  for  $\mu_a$ , and  $9.2\text{-}15.4 \text{ cm}^{-1}$  for  $\mu_s'$ . These experimental conditions must be kept in mind when considering our results reported in Section 3. In our experimental conditions, we found that a superficial layer up to 0.4 cm thick does not affect the accuracy of quantitative spectroscopy of the underlying material.

It has been suggested that three-layered media in a planar geometry may be taken as a rough model of the human head to predict the optical diffuse reflectance. In fact, the superficial layer, the clear layer, and the underlying block would mimic the scalp/skull, the cerebrospinal fluid, and the brain cortex, respectively.<sup>2</sup> However, our *in vivo* data on infant heads has not shown effects on the optical data similar to those observed in the three-layered media examined. Preliminary studies in spherical phantoms with the same layered structure (superficial layer on top, clear layer in the middle and spherical block in the bottom) show the same channeling effect observed in the planar geometry, so that a curved geometry does not significantly modify our results. An explanation for the failure of these simple models to describe the *in vivo* data on the infant head can be the fact that the CSF in the brain is not as geometrically homogeneous as the clear layer we made. A more irregular geometry, which includes regions where the clear layer is thinner, may reduce the light channeling effect. Furthermore, the CSF in the brain flows in a region which contains blood vessels, whose presence significantly contributes to light absorption.

#### 5. Conclusion

##### Two-layered media:

Our results show that a superficial layer (i.e. skin, subcutaneous fat) less than about 0.4 cm thick does not affect the quantitative optical study of the underlying medium by the frequency-domain (110 MHz) multi-distance method.

##### Three-layered media / infant head:

We stress that our results do not demonstrate that the clear cerebrospinal fluid does not affect the diffuse reflectance measured on the head. However, our results indicate that to describe the propagation of light in the human head, one should consider more accurate models than a uniform clear layer sandwiched between two turbid media.

#### 6. Acknowledgments

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## 7. References

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