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Time resolved reflectance measurements on layered tissues with strongly varying optical properties

Andreas H. Hielscher[1,2], Hanli Liu[3], L. Wang[1],
Frank K. Tittel[2], Britton Chance[3] and Steven L. Jacques[1]

[1]University of Texas M.D. Anderson Cancer Center,
Laser Biology Research Laboratory, Houston, Texas 77030

[2]Rice University, Dept. of Electrical and Computer Engineering,
Houston, Texas 77251-1892

[3]University of Pennsylvania, Dept. of Biochemistry and Biophysics,
Philadelphia, Pennsylvania 19104-6089

1. INTRODUCTION

Most biological tissues consist of layers with different optical properties. A few examples are the skin, the esophagus, the stomach and the wall of arteries. An understanding of how the light propagates in such layered systems, is a prerequisite for any light based therapy or diagnostic scheme. For example different methods like continuous light, time or frequency resolved reflectance measurements have been employed to determine the blood oxygenation status of the brain.[1,2,3] However, little attention has been paid to the fact that the brain is actually encapsulated by several layers of optically very different tissues (skin, skull, meninges). The arachnoid, a substructure of the meninges, is almost absorption and scattering free. On the other hand the capillary bed in the gray matter has a high content of strongly absorbing blood.

In this study we investigated the influence of these kind of layers on time resolved reflectance measurements. Experiments were performed on layered gel phantoms and the results compared to Monte Carlo simulations and diffusion theory. It is shown that when a low absorbing medium is situated on top of a high absorbing medium, the absorption coefficient of the lower layer is accessible if the differences in the absorption coefficient are only small. In the case of large difference the optical properties of the upper layer dominate the signal and shield information on the lowest layer. The degree of this shielding effect depends on layer thickness as well as optical properties.

In the case of an almost absorption and scattering free layer inbetween two normal tissues, an overall increase of the signal is visible. However, the overall shape of the curve is about preserved. The apparent scattering coefficient is slightly decrease, while the apparent absorption coefficient is unaltered.

2. TIME RESOLVED REFLECTANCE MEASUREMENTS

Time resolved reflectance measurements on tissues can be used to determine the absorption and reduced scattering coefficient (μ_a, μ_s') of tissues.[4] In this technique a pico second pulsed light source is used as an input signal and the reflectance is measured as a function of time at a distance of a few centimeter. A detailed description of the

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experimental set up, which uses the method of single photon counting, can be found elsewhere.[5]

Once the time resolved signal has been obtained, the absorption and scattering coefficient can be found by fitting a diffusion theory curve to the data. Assuming a semi-infinite medium the zero boundary condition one finds for the reflectance R measured at a distance r at time t (for a more detailed derivation see [4]):

$$R(r,t) = (4\pi c D)^{-3/2}\ t^{-5/2}\ \exp\left(-\frac{r^2 + (1/\mu_s')^2}{4 c D}\frac{1}{t}\right)\ \exp(-\mu_a c t) \qquad (1)$$

where μ_s' is the reduced scattering coefficient, $D = [3(\mu_a + \mu_s')]^{-1}$ the diffusion coefficient and c the speed of light in the medium. Eq. (1) can be linearized in μ_a and μ_s' by taking the natural logarithm of R and assuming $\mu_s' >> 1$:

$$\ln(R(r,t)) = \text{const} - 5/2 \ln(t) - \frac{a}{t}\mu_s' - (c t + \frac{a}{t})\mu_a \qquad (2)$$

$$\text{with } a = \frac{3(r^2)}{4c} \qquad (2a)$$

This provides a simple and fast linear fitting algorithm. Notice that for $t << 1$, μ_s' which is usually much larger than μ_a dominates the behavior of the reflectance signal. For $t >> 0$ on the other hand μ_a has the strongest influence.

It has to be emphasized that the expression given in Eq.(2) is derived for a semi-infinite medium with the so called zero boundary condition. This boundary conditions is an oversimplification and leads to a deviation of diffusion theory from the actual data at early times.[6] In general on can say that the closer the source detector separation the higher the overestimation of the scattering and underestimation of the μ_a. Using a more appropriate boundary condition leads to better results at early times, however for the price of complexity.[7] A linearization of Eq.(1) in μ_a and μ_s' is not possible anymore and more advanced and more time consuming fitting algorithms are required. As a rule of thumb we found that for the optical properties of interest, the diffusion theory with zero boundary condition can be considered accurate after ~500ps.[6] Thus fitting data only beyond this point leads to an accurate determination of especially of the absorption coefficient μ_a. In the case of blood oxygenation measurements of the brain, μ_a is actually the parameter of interest. Inaccuracies in the determination are in this case acceptable.

3. LAYERED TISSUE STRUCTURES

In medical situation one usually does not encounter a semi-infinite homogenous medium. The first approximation (semi-infinite) is actually acceptable, since for all practical purposes significant contribution to measurements come only from a few centimeters around the source. However, the second assumption (homogenous) is almost always not true. Tissues are more or less in homogenous. The strongest absorbers, like blood, are actually localized in blood vessels which are surrounded by low absorbing background tissues. Furthermore one frequently encounters layered tissue structures. Layers can be as

thin as a few μm (e.g. the epidermis) up to about a centimeter (e.g. skull). Details of the actual tissue structure are often unknown. The question arises: Can time resolved reflectance spectroscopy nevertheless yield some useful information? Can the simple linear fitting routine based on Eq.(2) still be applied and how have the results to be interpreted?

3.1. Low absorbing layer on high absorbing medium

An example is considered in the Fig. 1. Here a time resolved Monte Carlo simulation[8] of two homogenous media and one layered medium is shown. The layered medium is composite of the material which constitutes the two homogenous media. The 4 mm thick upper layer has a low absorption coefficient of $\mu_a = 0.01\ cm^{-1}$. The underlying bulk material has a twenty-time higher absorption coefficient. The scattering coefficient $\mu_{s'} = 10\ cm^{-1}$ is the same in both media. It can be seen that the time response of the layered tissue follows in the early time the curve (I) for the homogenous medium with lower absorption. The photons travel only in the upper layer and are not influenced by the lower, stronger absorbing bulk material. After roughly 150 ps curve (III) and (I) start to deviate. This indicates that photons which have reached the lower layer contribute to the signal. After ~500 ps the curve of the layered medium becomes parallel to the curve (II) of the homogenous medium with the optical properties of the underlying medium. As discussed earlier, the absorption coefficient of a tissue mostly influences the late part of the time resolved reflectance. Thus a fit of diffusion theory (Eq.(2)) to curve (II) (homogenous medium) and curve (III) (layered medium) yields the same μ_a. This suggests, that one can measure through the upper layer the absorption coefficient μ_a of the hidden tissue. The layered structure of the tissue seems only to affect the apparent scattering coefficient.

This might be surprising, on the first view. However, if one considers that after 1 ns the photons actually have traveled ~22 cm in the tissue (n=1.37). This means that by far most of the time a photon propagates, it spends in the lower layer. The 4 mm thick upper layer becomes optically thinner and thinner the more time elapses.

In Figure 2 another Monte Carlo simulation is shown. The absorption coefficient of the lower layer has been increased ten times to $\mu_a = 2\ cm^{-1}$ and is thus 200 time higher than the μa of the upper layer. Now the slope of the time resolved impulse response measured on layered system is not any longer parallel to the homogenous medium with the high absorption. Thus, a diffusion theory fit gives different scattering and absorption coefficient for both system.

The results of the simulations were also tested experimentally on layered tissue phantoms, made out of collagen gels. $Ti0_2$ powder was added to the gel to introduce scatterers into the medium. India ink in different concentrations served as absorber. Gels with different optical properties were stacked on top of each other to yield a layered tissue structure. As a light source a pulsed laser diode with a wavelength of 780 nm and a pulse with of ~50 ps was used. The findings from Monte Carlo simulations could be confirmed as shown in figure 3. Through the upper layer one can measure the absorption coefficient of the underlying medium, by fitting the late part of the time resolved reflectance if the difference in absorption coefficient is not to high. Different thicknesses of the upper layer seem only to affect the amplitude of the signal, but not the determination of the absorption coefficient of the lower layer. In Fig.4 the absorption coefficient of the lower layer has been

increased, and it is not possible anymore to determine the absorption coefficient of the underlying tissue.

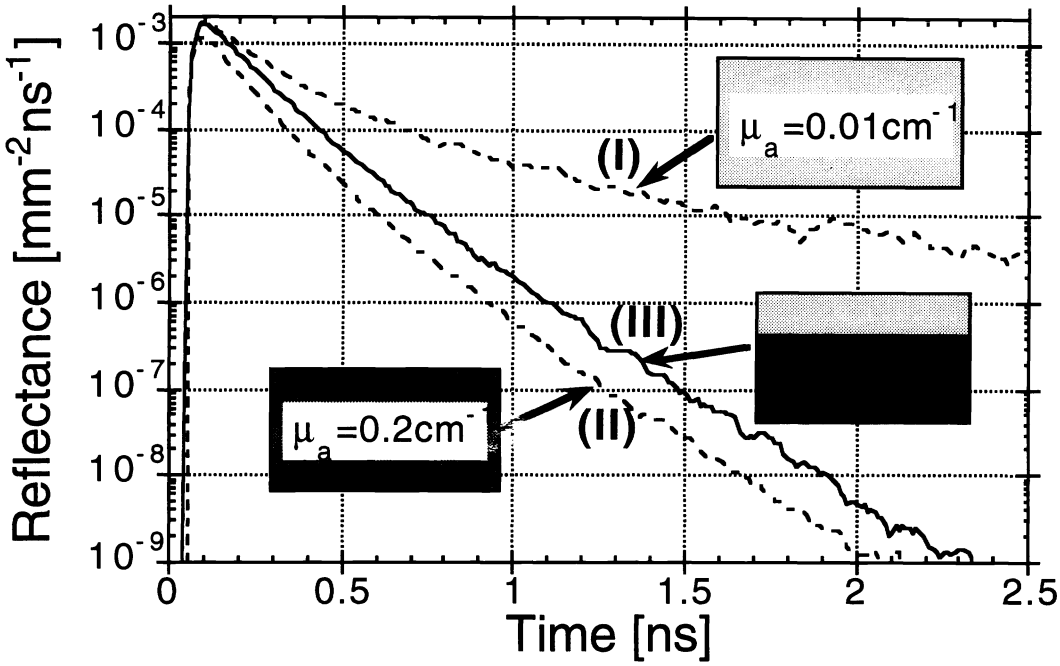

Fig. 1: Monte Carlo Simulations for a two layered system. Both layers have the same μ_s'. The upper layer is 4mm thick. The source-detector separation is 1cm. The related homogenous media are shown as reference.

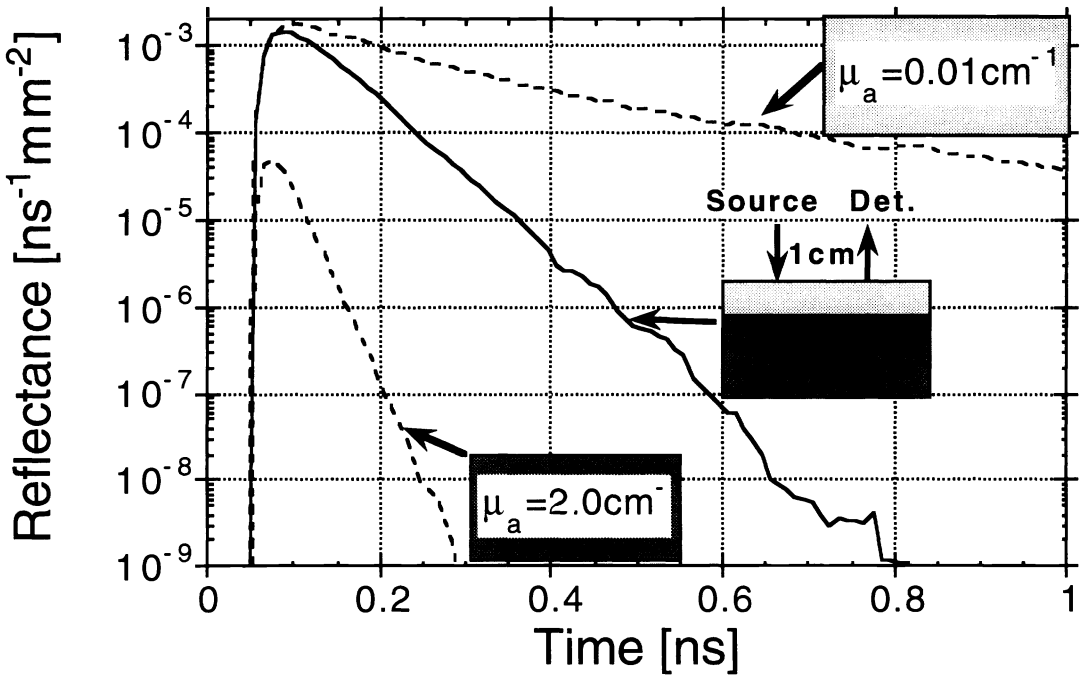

Fig. 2: Monte Carlo Simulations for a two layered system. All parameters as in Fig.1, only the absorption coefficient of the lower layer has been increased 10 times.

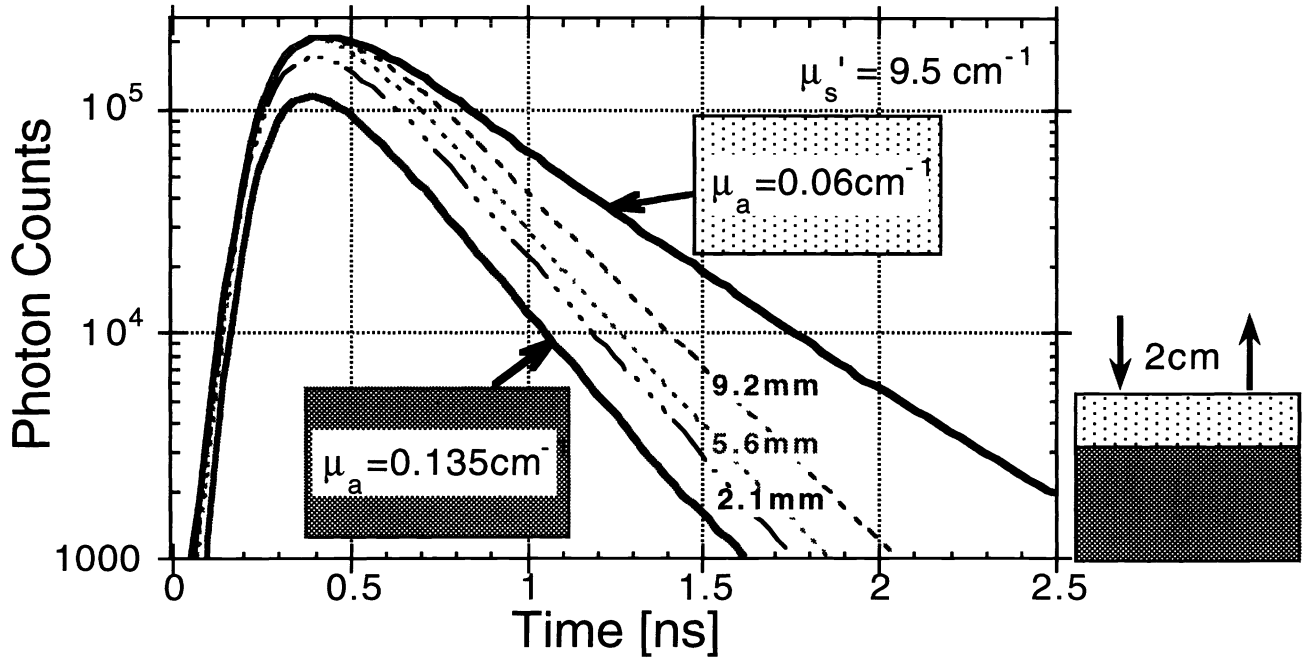

Fig.3: Experiments on layered gel phantoms with a layer of low absorbing gel on top of a ~twice as strong absorbing gel. The values labeled along the dashed curves indicate the thicknesses of the top layer.

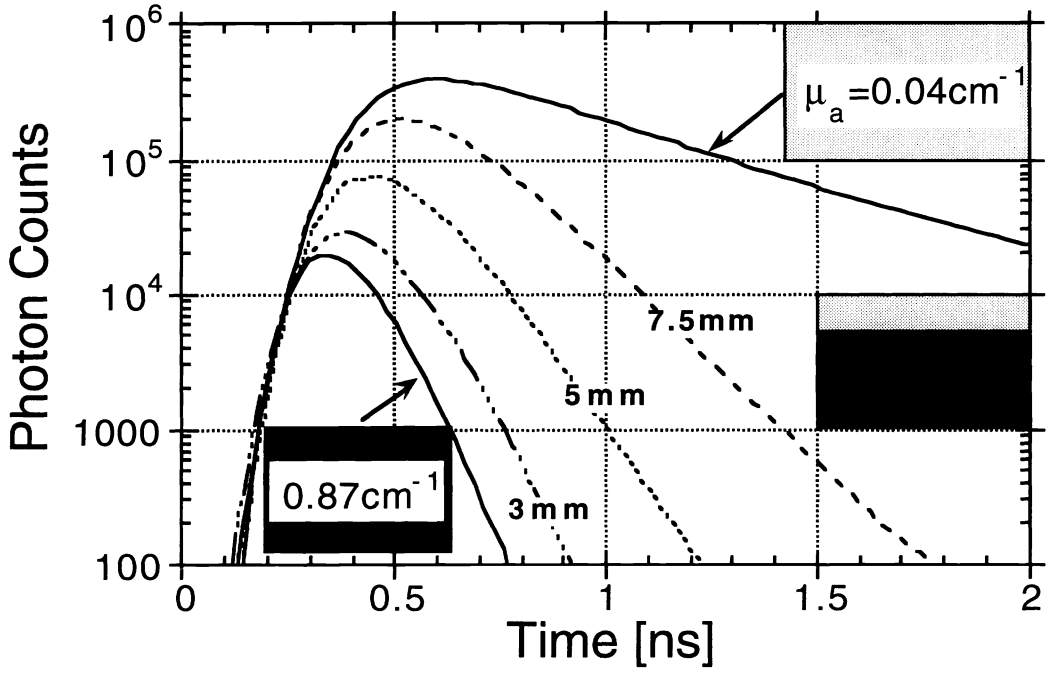

Fig.4: Experiments on layered gel phantoms with a layer of low absorbing gel on top of a very high absorbing gel. The values labeled along the dashed curves indicate the thicknesses of the top layer. The scattering coefficient μ_s' is 12.0 cm^{-1} for all media.

In Fig.5 the results of 12 experiments on layered gel phantoms are summarizes. On the x-axes the absorption coefficient of the underlying medium, is indicated. For 2 different layer thicknesses the apparent absorption coefficient of this layered tissue structure was determined by fitting Eq.(2) to the measured time resolved reflectance curve. The source detector separation was 2cm. The absorption coefficient of the upper layer was $\mu_a = 0.042\ cm^{-1}$. As can be seen if the upper layer is thin (3 mm), the apparent absorption coefficient matches the absorption coefficient of the lower layer within the accuracy of the measurements up to ~0. 5 cm^{-1}. If the absorption coefficient of the underlying material is further increased, the apparent absorption coefficient underestimates the actual absorption coefficient.

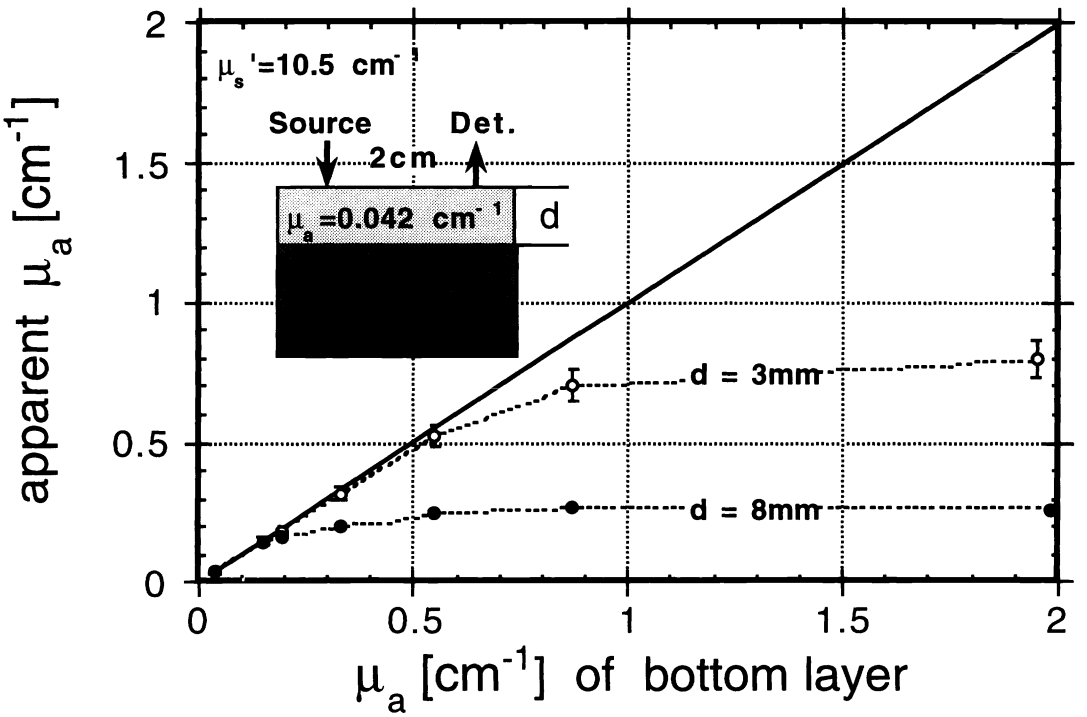

Fig.5: The apparent absorption coefficient as a function of the absorption coefficient of the underlying medium and layer thickness.

If the layer thickness increased the deviation between actual and apparent absorption coefficient occurs at even lower absorption coefficient of the bottom medium (here at $\mu_a = 0.2cm^{-1}$). If the absorption of underlying tissue is increased beyond ~1 cm^{-1}, the system with the 8 mm layer does not show any measurable change in the apparent absorption coefficient. The top layer "shields" the information about the underlying tissue. This result is particular important for the case of blood oxygenation measurement on the brain. Here it is often desirable to detect oxygenation changes, which result in a change of the blood absorption coefficient [1-3]. If the absorption coefficient of the brain tissue is already higher than 0.2, changes in the oxygenation status may not be detectable. First results of absorption measurements on the brain show an apparent absorption coefficient of 0.152 cm^{-1} (see Fig.6). Further studies are necessary to determine if the this is only due to the low absorbing skull or if actually the underlying tissue has this low absorption coefficient.

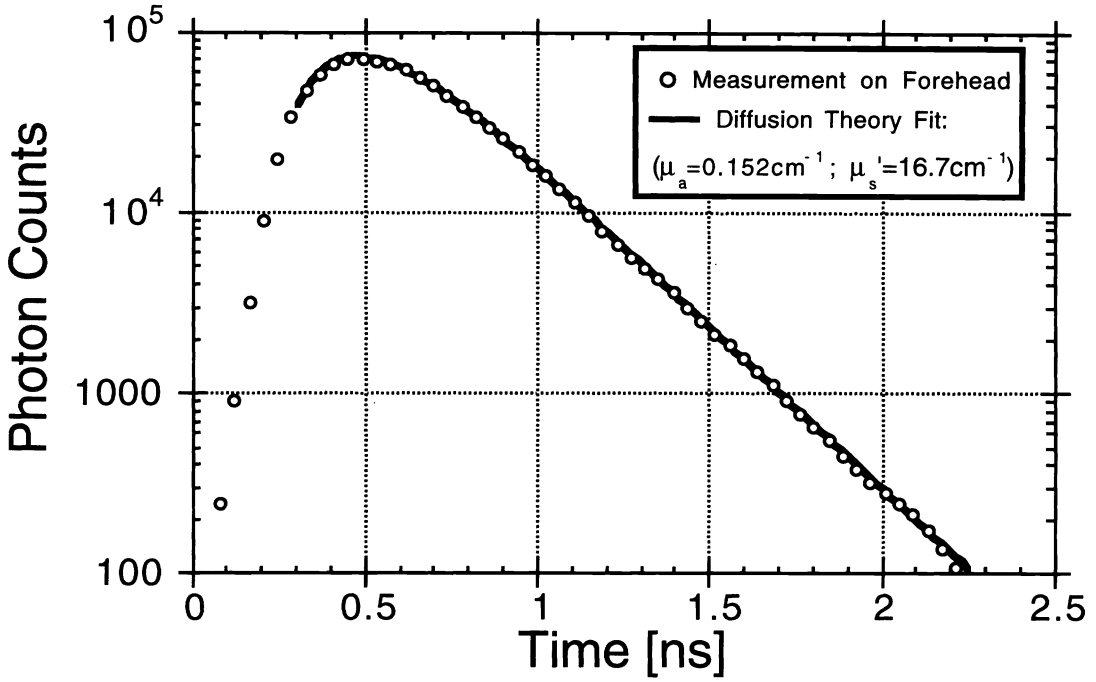

Fig.6: In vivo measurement on the forehead. The source detector separation was 1.9 cm. A wavelength of λ=830nm was used.

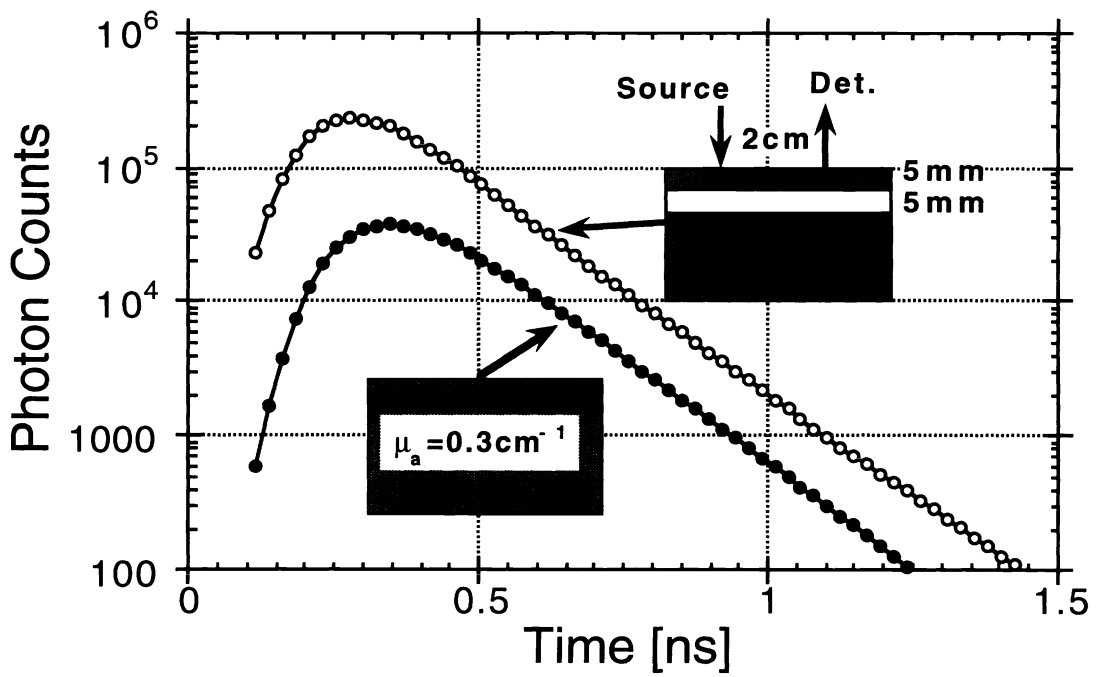

Fig.7: Influence of an almost absorption and scattering free layer on the time resolved impulse response.

3.2. Scattering and absorption free layers

As already mentioned in the introduction, one also encounters layers with almost no absorption and scattering in biological tissues. The arachnoid, a sub layer of the meninges, which encapsulates the brain, is such a layer. The sub arachnoid is filled with brain fluid. The influence of such a layer on time resolved reflectance measurements was also investigated.

Fig.7 shows the results of an experiment on a layered gel phantom. A pure Gel layer, with no TiO_2 or india ink was sandwiched between a bulk medium with $\mu_a = 0.3cm^{-1}$ and an upper 4 mm layer with the same absorption coefficient. ($\mu_s' = 10cm^{-1}$). The source detector separation was 2 cm. As can be seen the only difference in the measurements are that the overall amplitude of the signal has been increased and the maximum has been slightly shifted towards zero. The absorption coefficient is unchanged. Thus it can be conclude that the sub-arachnoid does not effect the absorption measurements s of the blood in the brain

4. SUMMARY

In this study the influence of layered tissue structures on time resolved reflectance measurements were investigated experimentally and numerically. Layered gels with different concentration of TiO_2 and india ink were used as phantoms, which simulate for example the skull/brain situation. It was demonstrated that when the difference in the optical properties are small, the absorption coefficient of the lower layer can be determined. In the case of large difference the optical properties of the upper layer dominate the signal and shield information on the lowest layer. The degree of this shielding effect depends on the layer thickness as well as the optical properties.

Furthermore the case of an almost absorption and scattering free layer inbetween two normal tissues was examined. An important example for this kind of layer is the arachnoid a sub layer of the meninges which encapsulate the brain. Experimental results show that absorption and scattering free layers give rise to an overall increase of the signal. However, the shape of the curve is only changed slightly. The apparent scattering coefficient is decrease, while the apparent absorption coefficient seems to be unaltered.

5. ACKNOWLEDGMENT

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