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Non-invasive determination of muscle blood flow in the extremities from laser Doppler spectra

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Abstract

We investigate theoretically the non-invasive determination of blood flow in muscles of the extremities using laser Doppler measurements. Laser Doppler spectra are calculated using Monte Carlo simulations and solutions of the correlation diffusion equation. The extremities are modelled as a two-layered turbid medium. The first layer represents the skin and subcutaneous fat layer and the second layer the muscle. It is shown that the absolute root-mean-square velocity of the blood in the muscle layer can be accurately derived in many practical cases if the laser Doppler spectra are measured at a distance which is sufficiently far from the source, and if the optical properties of the muscle are simultaneously determined.

1. Introduction

In recent years great efforts have been made to determine the concentration of oxy- and deoxyhaemoglobin in tissue by near-infrared spectroscopy. Measurements in the steady-state, frequency and time domains were performed on different parts of the human body, especially the extremities and the head. These experiments enable the haemodynamics of muscle and brain during different forms of exercise, stimulation and disease to be studied non-invasively (Yodh and Chance 1995). However, for a complete description of the haemodynamics in these organs the blood perfusion has to be known. Thus, in addition to the concentration, the velocity of the blood cells has to be determined. Laser Doppler measurements are a suitable method for performing the latter task.

Laser Doppler measurements make use of the Doppler shifts of the photons caused by their scattering on moving particles during their propagation in the turbid medium (Stern 1975). The Doppler shifts can be measured by a photodetector due to beating with the unshifted photons. As a result of the scattering in the turbid medium and the random flow directions of the blood, light is incident on the erythrocytes from all directions and many different Doppler shifts occur, producing a Doppler spectrum. Light may also undergo multiple Doppler scattering before it is detected.

Laser Doppler experiments are normally performed to measure the microvascular blood perfusion (Nilsson *et al* 1980, Bonner and Nossal 1981, Leahy *et al* 1999). Thus, the distance ρ

between the laser source and the detection is small ($\rho < 1$ mm), and therefore the volume probed by the detected photons is accordingly small. Although laser Doppler measurements have been reported in a large number of publications, the method has not found widespread clinical application. The main reason for this is that the available instruments deliver only tissue perfusion values in relative units. It is also difficult to separate the contribution of a change in the blood concentration or in the blood velocity to a measured change in the perfusion (Leahy *et al* 1999). In this paper we show that these problems can be solved by measuring the optical properties of the investigated tissue simultaneously with the laser Doppler measurements. Knowledge of the optical properties at several wavelengths (two wavelengths if oxy- and deoxyhaemoglobin are the only essential absorbers) allows one to calculate the concentration of oxy- and deoxyhaemoglobin in the tissue. Knowing the blood concentration and the optical properties of the investigated tissue at the considered wavelength, the laser Doppler spectra can be calculated by solving the correlation transport equation or its approximation, the correlation diffusion equation. Absolute quantities for the velocity of the blood cells can then be obtained by fitting the calculated laser Doppler spectra to the measured spectra. However, it is difficult to apply this method to the measurement of microvascular blood perfusion in the skin, because the heterogeneity of skin and the small volumes of tissue investigated strongly complicate the determination of the optical coefficients.

Measurements on large tissue volumes using large distances between the source and the detector simplify the determination of the optical properties and allow the assumption that the different tissues (for example, skin or muscle) can be regarded as homogeneous. The easiest situation occurs if the probed tissue volume can be regarded as homogeneous and semi-infinite. In this case the optical properties can be readily determined (Patterson *et al* 1989, Farrell *et al* 1992, Andersson-Engels *et al* 1993). Thus, to determine the absolute blood velocity all we need is a fast and accurate method to calculate laser Doppler spectra of semi-infinite homogeneous turbid media (sections 3.1–3.4 in this paper).

Some researchers have investigated blood perfusion in large tissue volumes by measurements using large distances between the laser source and detector ($\rho > 10$ mm) (Boas *et al* 1995, Lohwasser and Soelkner 1999). Possible applications are the non-invasive measurement of blood perfusion in the brain or of muscles in the extremities. These tissues are heterogeneous and, thus, part of the laser Doppler spectrum stems from the layers lying above the muscle or brain tissue. Therefore, it has to be investigated whether it is still possible to apply a simple semi-infinite homogeneous model to deduce the tissue blood perfusion in these cases (section 3.5 in this paper).

In the present study laser Doppler spectra were calculated using Monte Carlo simulations and with solutions of the diffusion correlation equation. (We showed that Monte Carlo simulations accurately describe experimental laser Doppler spectra (Kienle *et al* 1996).) Both methods were compared with each other for semi-infinite homogeneous and two-layered turbid media, and the conditions when the solutions of the correlation diffusion equation are a good approximation to Monte Carlo simulations were investigated. The influence of the anisotropy factor on the laser Doppler spectra was examined, as was the performance of two different Monte Carlo methods for calculation of the laser Doppler spectra.

The possibility of obtaining the absolute root-mean-square velocity of the blood in the muscle of the extremities was investigated by fitting the solution of the correlation diffusion equation for a semi-infinite homogeneous medium to the solution of the two-layered turbid medium, which represents the extremity, assuming that the optical coefficients of the second layer (muscle layer) are known. The nonlinear regressions were performed for different blood velocities in the second layer of the two-layered medium to simulate haemodynamics measurements on the muscle during, for example, exercise or stimulation experiments.

2. Theory

The Monte Carlo method and solutions of the correlation diffusion equation were used to calculate laser Doppler spectra from turbid media. The first technique is within its statistical nature an exact solution of the correlation transport equation and the latter is an approximation to the correlation transport equation (Boas 1996). A semi-infinite homogeneous geometry and a semi-infinite two-layered geometry were considered. The latter consists of a first layer with thickness l and an infinitely thick second layer. It was assumed that the blood in the turbid media has a random flow (isotropic velocity distribution) and, if not stated otherwise, that the velocity distribution for the moving scatterers is Gaussian.

We note that for medical applications the dynamic properties of turbid media are mostly measured in the frequency domain (laser Doppler spectra), whereas for other applications the measurements in the time domain (correlation measurements) prevail (Pine *et al* 1988, MacKintosh and John 1989, Heckmeier *et al* 1997).

2.1. Monte Carlo method

The correlation transport equation was solved using the Monte Carlo method. The main features of the code are similar to the Monte Carlo simulations for solving the radiative transport equation in turbid media containing only static scatterers (Wilson and Adam 1983). The Henyey–Greenstein function was used as phase function. In addition to the calculation of the propagation of light in a static turbid medium, at each interaction point the probability was calculated that the photon is scattered by a moving particle (Stern 1985, Koelink *et al* 1994, Mul *et al* 1995, Kienle *et al* 1996). The probability of photon scattering at the moving particles related to all scattering processes (static and dynamic) in the turbid media P_b is the product of the concentration of the blood c_b (given in vol.%) and the quotient of the scattering coefficient of blood $\mu_{s,\mathrm{blood}}$ and the total scattering coefficient μ_s of the considered turbid medium: $P_b = c_b \mu_{s,\mathrm{blood}}/\mu_s$. At each scattering at a moving particle the Doppler Δf shift was calculated using

$$\Delta f = 2 f_0 \frac{\Delta\vec{k}\vec{v}}{c|\Delta\vec{k}|} \sin(\theta/2) \tag{1}$$

$$= \frac{2|v|}{\lambda/n} \cos(\delta) \sin(\theta/2) \tag{2}$$

where f_0 is the frequency and λ the wavelength of the incident photon, θ the scattering angle, $\vec{v}$ the velocity of the moving particle, c the velocity of light in the turbid medium and $\Delta\vec{k}$ the difference of the scattered and incident wavevectors (Jentink *et al* 1990). The angle between $\Delta\vec{k}$ and the velocity of the scattering particle is termed δ. When a photon is scattered more than once at a moving particle, all frequency shifts are added to obtain the total frequency shift of the considered photon. For the re-emitted photons the total frequency shift was scored as a function of the distance between the location of the reflectance and the incident source. The laser Doppler spectra $S(f)$ were obtained by calculating a sufficiently large number of photons (typically 10^7). Alternatively to the method described above, which calculates $S(f)$ directly in the frequency domain, the Monte Carlo technique was also used to obtain the laser Doppler spectra via calculation of the autocorrelation function $g(\tau)$ and Fourier transforming $g(\tau)$. A detailed description of this approach can be found in the literature (Boas 1996). Briefly, as in the direct method, propagation of light through the turbid medium was calculated. However, instead of computing the Doppler shift at each scattering interaction with the moving particle, the velocity of the moving scatterers was not considered and only the dimensionless momentum transfer $Y = \sum_{j=1}^{n} 2\sin^2(\theta_j/2)$ was calculated, where θ_j is the scattering angle of the jth scattering on a moving particle of the considered photon path. The probability distribution for $P(Y)$ was obtained by storing the Y values for a large number of photons. From $P(Y)$ the autocorrelation function $g_M(\tau)$ was calculated assuming a Gaussian velocity distribution

$$g_M(\tau) = \int_0^\infty P(Y) \exp\left(-\frac{1}{3} Y k^2 \langle \Delta r^2(\tau) \rangle\right) \mathrm{d}Y \tag{3}$$

where $\langle \Delta r(\tau) \rangle$ is the displacement of the moving particle in time τ and k is the modulus of the wavevector. For random flow we have $\langle \Delta r^2(\tau) \rangle = \langle v^2 \rangle \tau^2$, where $\langle v^2 \rangle$ is the mean square velocity of the moving particles ($v_{\mathrm{rms}} = \sqrt{\langle v^2 \rangle}$). The Fourier transform of $g_M(\tau)$ delivers the laser Doppler spectrum $S(f)$. In section 3.1 we compare the laser Doppler spectra calculated with the direct Monte Carlo simulation and with the approach using the autocorrelation function.

2.2. *Correlation diffusion equation*

The disadvantage of the Monte Carlo simulations is the long calculation times needed to compute low-noise laser Doppler spectra. The correlation diffusion equation delivers analytical solutions for simple geometries, from which the laser Doppler spectra can be calculated much faster. However, it is an approximation to the correlation transport equation, and its validity has to be verified for the considered application. A derivation of the correlation diffusion equation can be found in the literature (Boas 1996). For a homogeneous medium and a point source at $\vec{r}_0$ we have

$$(D\nabla^2 - \mu_a - \tfrac{1}{3} P_b \mu_s' k^2 \langle \Delta r^2(\tau) \rangle) g_D(\vec{r}, \tau) = -\delta(\vec{r} - \vec{r}_0) \tag{4}$$

where $D = [3(\mu_s' + \mu_a)]^{-1}$ is the photon diffusion coefficient, μ_a is the absorption coefficient, and ∇^2 is the Laplace operator. Equation (4) shows that the correlation diffusion equation has, besides μ_a, another loss term $(\frac{1}{3} P_b \mu_s' k_0^2 \langle \Delta r^2(\tau) \rangle)$, which represents the loss of correlation due to the dynamics of the scattering processes. Thus, the solutions known for the steady-state diffusion equation can be used to solve the correlation diffusion equation simply by replacing μ_a by $\mu_a + \frac{1}{3} P_b \mu_s' k_0^2 \langle \Delta r^2(\tau) \rangle$. The laser Doppler spectra were obtained by Fourier transforming $g_D(\tau)$.

In this study equation (4) was solved for semi-infinite turbid media. The extrapolated boundary condition and calculations of the reflectance as the integral of the radiance over the backward hemisphere (Haskell *et al* 1994) were applied, because this solution is closer to Monte Carlo simulations compared with other solutions for mismatched boundary conditions (Kienle and Patterson 1997). Solutions of a semi-infinite homogeneous geometry (Kienle and Patterson 1997) and a semi-infinite two-layered geometry (Kienle *et al* 1998) were used.

3. Result

Sections 3.1–3.4 show laser Doppler spectra from semi-infinite homogeneous turbid media. For all calculations we used the following optical coefficients, which are typical for biological tissue in the near-infrared wavelength range: $\mu_s' = 1\ \mathrm{mm}^{-1}$, $\mu_a = 0.01\ \mathrm{mm}^{-1}$, anisotropy factor $g = \langle \cos(\theta) \rangle = 0.8$, refractive index $n = 1.4$. Section 3.5 gives results of the investigation to obtain the absolute root-mean-square velocity of the second layer of a semi-infinite two-layered turbid medium representing the muscle in the extremities.

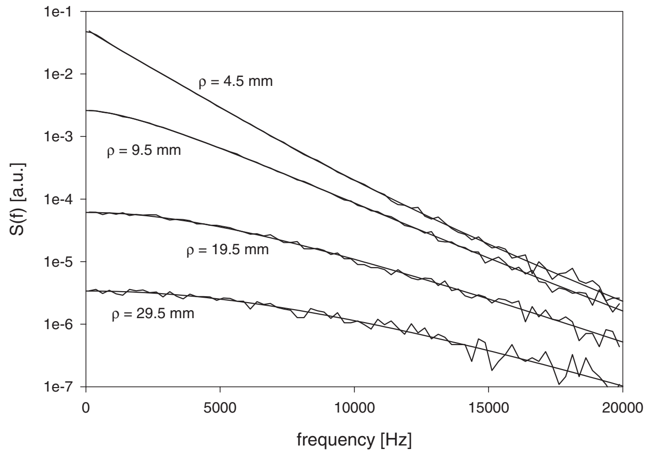

Figure 1. Comparison of laser Doppler spectra from semi-infinite homogeneous turbid media calculated using Monte Carlo simulations directly in the frequency domain (zigzag line) or via the autocorrelation function (smooth line). The optical properties are $\mu_s' = 1\ \mathrm{mm}^{-1}$, $\mu_a = 0.01\ \mathrm{mm}^{-1}$, $g = 0.8$ and $n = 1.4$. The root-mean-square velocity is $v_{\mathrm{rms}} = 1\ \mathrm{mm\ s}^{-1}$ and $P_b = 0.1$.

3.1. Laser Doppler spectra calculated using two different Monte Carlo methods

As stated in section 2, laser Doppler spectra can be calculated with the Monte Carlo method directly in the frequency domain or using the autocorrelation function and the Fourier transform technique. Figure 1 compares laser Doppler spectra derived by the two approaches for a semi-infinite homogeneous turbid medium at different distances from the incident source. Equal numbers of photons were used for both simulations. As figure 1 indicates, it can be shown that the calculation of the laser Doppler spectra via the autocorrelation function delivers smaller uncertainties, because fewer random variables are involved in the simulations: the modulus and the direction of the velocity of the scatterers are not sampled but are assumed to be Gaussian and isotropic respectively. The disadvantage of this method is that it delivers laser Doppler spectra only for these assumptions. We compared laser Doppler spectra calculated with Monte Carlo simulations directly in the frequency domain using different non-Gaussian velocity distributions having the same v_{rms}. It was found that the different distributions strongly influence the spectra provided that the number of scatterings of the photons with the moving particles is small. For large distances and large P_b, however, the spectra converge into the same curves (figure not shown). If not stated otherwise, the Monte Carlo method using the autocorrelation function and the Fourier transform technique was used in the present study.

3.2. Dependence of laser Doppler spectra on the absorption and scattering coefficients of turbid media

In this subsection we show the dependence of laser Doppler spectra on the optical properties. Figure 2 shows laser Doppler spectra from homogeneous semi-infinite turbid media at two distances from the source ($\rho = 4.5$ mm and $\rho = 19.5$ mm). Increasing the absorption coefficient causes a decrease of $S(f)$ at all frequencies because the probability that a photon is re-emitted decreases for all numbers of interactions (i.e. Doppler shifts). Decreasing the reduced scattering coefficient increases $S(f)$ for small f and decreases $S(f)$ for large f, because the re-emitted photons at a certain distance have, on average, experienced fewer Doppler shifts.

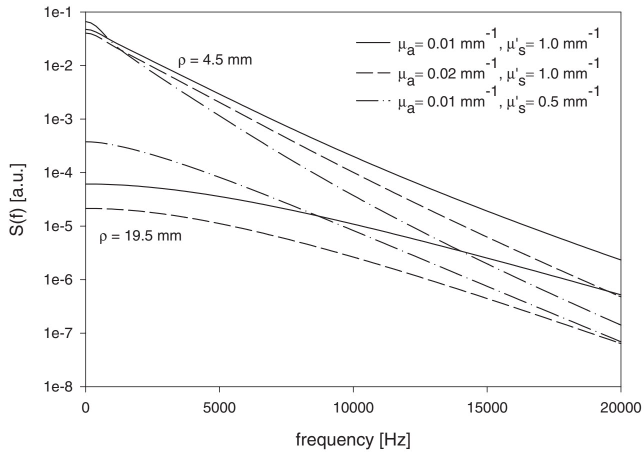

Figure 2. Laser Doppler spectra from semi-infinite homogeneous turbid media calculated using the solution of the correlation diffusion equation for different absorption and reduced scattering coefficients as indicated in the legend. The refractive index is $n = 1.4$. The root-mean-square velocity is $v_{\mathrm{rms}} = 1$ mm s^{-1} and $P_b = 0.1$.

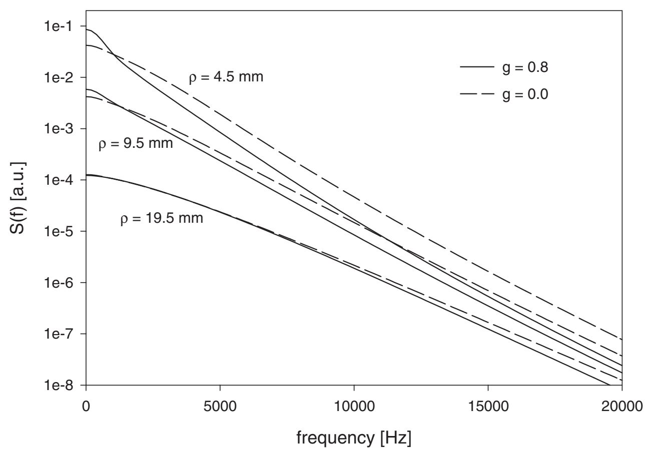

Figure 3. Laser Doppler spectra from semi-infinite homogeneous turbid media calculated using the Monte Carlo method for different g factors. The optical properties are $\mu_s' = 1$ mm^{-1}, $\mu_a = 0.01$ mm^{-1} and $n = 1.4$. The root-mean-square velocity is $v_{\mathrm{rms}} = 1$ mm s^{-1} and $P_b = 0.03$.

3.3. Dependence of the laser Doppler spectra on the anisotropy factor

It is well known that light propagation in turbid media is approximately independent of the anisotropy factor g provided that the reduced scattering coefficient μ_s' is constant and the reflectance is measured far from the incident source ($\rho \gg (\mu_s')^{-1}$). This principle is known as the similarity relation (Wyman *et al* 1989, Bevilacqua and Depeursinge 1999). We investigated the dependence of laser Doppler spectra on the anisotropy factor. Figures 3 and 4 show laser Doppler spectra calculated using two different anisotropy factors ($g = 0$ and $g = 0.8$) for $P_b = 0.03$ and $P_b = 0.1$ respectively. Similar to the light propagation in static tissue, the figures show that the laser Doppler spectra calculated with different g factors show better agreement the larger the distances are between the source and detector. However, the differences are

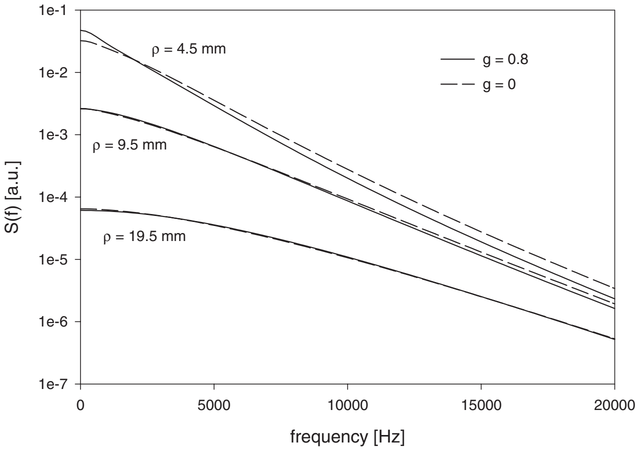

Figure 4. Laser Doppler spectra from semi-infinite homogeneous turbid media calculated with the Monte Carlo method are shown for different g factors. The optical properties are $\mu_s' = 1$ mm^{-1}, $\mu_a = 0.01$ mm^{-1} and $n = 1.4$. The root-mean-square velocity is $v_{\mathrm{rms}} = 1$ mm s^{-1} and $P_b = 0.1$.

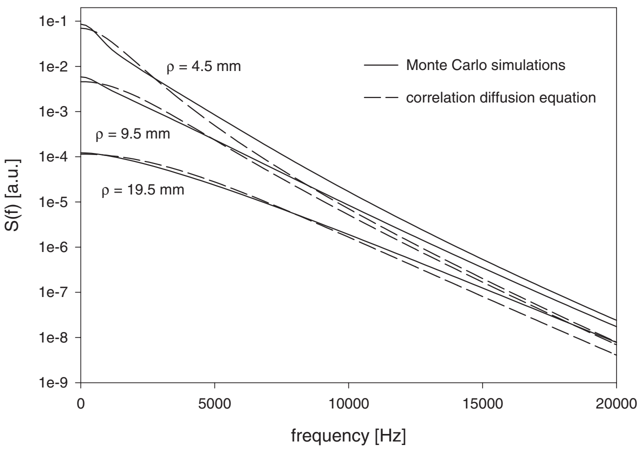

Figure 5. Comparison of laser Doppler spectra from semi-infinite homogeneous turbid media calculated using Monte Carlo simulations and solutions of the correlation diffusion equation. The optical properties are $\mu_s' = 1$ mm^{-1}, $\mu_a = 0.01$ mm^{-1}, $n = 1.4$ and $g = 0.8$. The root-mean-square velocity is $v_{\mathrm{rms}} = 1$ mm s^{-1} and $P_b = 0.03$.

strongly influenced by the concentration of the moving scatterers, or more precisely by P_b. The greater P_b, the better is the agreement, because the light has experienced more interactions with the moving scatterers and, thus, the similarity relation is better fulfilled.

3.4. Comparison of Monte Carlo simulations and solutions of the correlation diffusion equation

Figures 5 and 6 compare the laser Doppler spectra calculated with the Monte Carlo method with the solution of the correlation diffusion equation for semi-infinite homogeneous turbid media. The same parameters are used in both figures except that P_b is varied ($P_b = 0.03$ in figure 5 and $P_b = 0.1$ in figure 6). Both figures show that diffusion theory agrees better with

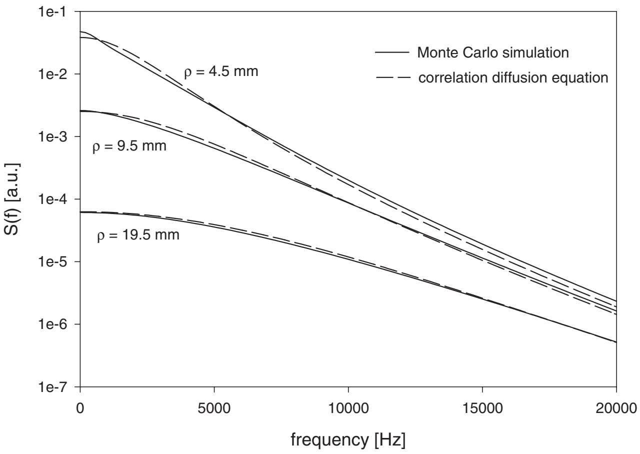

Figure 6. Comparison of laser Doppler spectra from semi-infinite homogeneous turbid media calculated using Monte Carlo simulations and solutions of the correlation diffusion equation. The optical properties are $\mu_s' = 1\ \mathrm{mm}^{-1}$, $\mu_a = 0.01\ \mathrm{mm}^{-1}$, $n = 1.4$ and $g = 0.8$. The root-mean-square velocity is $v_{\mathrm{rms}} = 1\ \mathrm{mm\ s}^{-1}$ and $P_b = 0.1$.

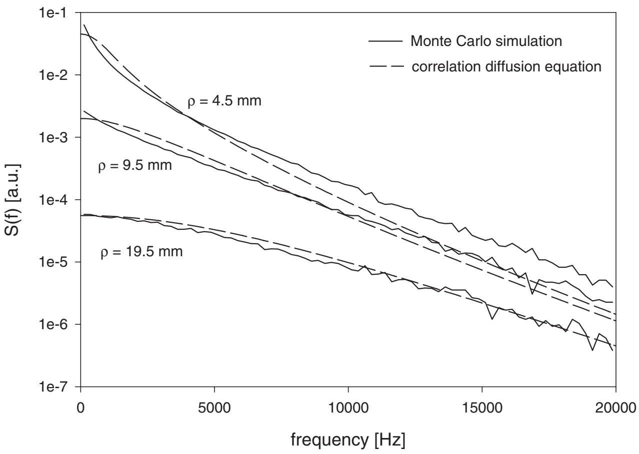

Figure 7. Comparison of laser Doppler spectra calculated using Monte Carlo simulations (directly calculated in the frequency domain) and solutions of the correlation diffusion equation for a two-layered turbid medium. The optical properties are $\mu_{s1}' = 1.2\ \mathrm{mm}^{-1}$, $\mu_{a1} = 0.01\ \mathrm{mm}^{-1}$, $\mu_{s2}' = 0.5\ \mathrm{mm}^{-1}$, $\mu_{a1} = 0.02\ \mathrm{mm}^{-1}$, $g = 0.8$, and $n = 1.4$. The root-mean-square velocities of the first and the second layers are $v_{\mathrm{rms1}} = 1\ \mathrm{mm\ s}^{-1}$ and $v_{\mathrm{rms2}} = 2\ \mathrm{mm\ s}^{-1}$, respectively, and $P_{b1} = 0.02$, $P_{b2} = 0.1$. The thickness of the first layer is $l = 2$ mm.

Monte Carlo simulations the larger the distances are between the source and the detector. This feature is well known for light propagation in turbid media containing no moving particles. Similar to section 3.3, however, P_b also has an important impact. For $P_b = 0.1$ the agreement is considerably better than for $P_b = 0.03$, because the number of interactions of the photons with the moving scatterers is larger for $P_b = 0.1$.

Figure 7 compares laser Doppler spectra calculated using Monte Carlo simulations and the solution of the diffusion correlation equation for semi-infinite two-layered turbid media. The optical properties of the two layers were chosen to resemble those of muscle in the extremities (second layer) and those of the layers above the muscle layer, namely the skin and the subcutaneous fat layer (first layer) (Kienle and Glanzmann 1999). As in the homogeneous case, the agreement between the two theories is better the larger the distance ρ is. The absolute differences are similar to those found for homogeneous turbid media considering that the average blood concentrations of the two layers of the two-layered medium are between those of the homogeneous media shown in figure 5 and 6.

3.5. Determination of the root-mean-square velocity of the blood of the muscle layer

In order to investigate if it is possible to obtain the root-mean-square velocity of the blood cells of the muscle layer in the extremities using the solution of the semi-infinite homogeneous geometry, we performed laser Doppler spectra of two-layered media representing the muscle (second layer) and the layers above the muscle layer (first layer). These spectra served as 'experimental data', to which laser Doppler spectra from a semi-infinite homogeneous turbid medium were fitted using the optical properties of the muscle layer of the two-layered turbid medium as the optical properties of the homogenous turbid medium. It was also assumed that the blood concentration of the muscle layer c_b is known. (The optical properties of muscle and the concentration of blood can be determined by simultaneous measurements of near-infrared spectra in the time or frequency domains; see section 4.) The root-mean-square velocity and a scaling constant of the y-axis were the fitting parameters in the nonlinear regression. We used the corresponding solutions of the correlation diffusion equation for the calculations of the laser Doppler spectra of the two-layered and the semi-infinite homogeneous turbid media. The root-mean-square velocity of the blood in the second layer was varied ($v_{\mathrm{rms2}} = 0.5, 1.0, 2.0, 3.0$ mm s^{-1}). Figure 8 shows the root-mean-square velocities v_{rms} obtained from the nonlinear regression for different ρ. The thickness of the first layer was 2 mm. For $\rho = 29.5$ mm the root-mean-square velocities obtained from the nonlinear regressions have differences less than 20% compared with v_{rms2}. For smaller distances the errors are larger, because the probability that the detected photons have propagated through the second layer is smaller. Figure 9 shows the results from similar nonlinear regressions, but the thickness of the first layer is increased to $l = 5$ mm. As expected, the differences between the estimated v_{rms} and the v_{rms2} are greater than the differences found for $l = 2$ mm, but they are still less than 20% for $\rho = 29.5$ mm.

In the nonlinear regressions shown in figures 8 and 9 it was assumed that the optical properties of the muscle are known from independent measurements. The absorption coefficient of the muscle on the forearm can be accurately obtained from measurements in the time or frequency domains, if the thickness of the layers above the muscle is less than about 4 mm (Franceschini *et al* 1998, Kienle and Glanzmann 1999). However, the accuracy of the obtained scattering coefficient is worse (Kienle and Glanzmann 1999). Figure 10 shows the results of nonlinear regressions as shown in figure 8, but the results for the two-layered turbid media ('the experimental data') were calculated using $\mu'_{s2} = 0.7$ mm^{-1} instead of $\mu'_{s2} = 0.5$ mm^{-1}, and $\mu'_s = 0.5$ mm^{-1} was used for the homogeneous turbid medium in the nonlinear regression routine. Thus, it was assumed that the reduced scattering coefficient of the muscle was determined with an error of about 30%. For $\rho = 19.5$ and 29.5 mm the differences between the estimated v_{rms} and the v_{rms} of muscle are greater than those shown in figure 8 (by up to about 35%). For $\rho = 9.5$ mm the differences are smaller for large v_{rms2}. This latter behaviour is probably caused by two different effects. The first due to the relatively small penetration depth and the second due to the fact that the wrong μ'_s values used in the nonlinear regressions seem to cancel each other.

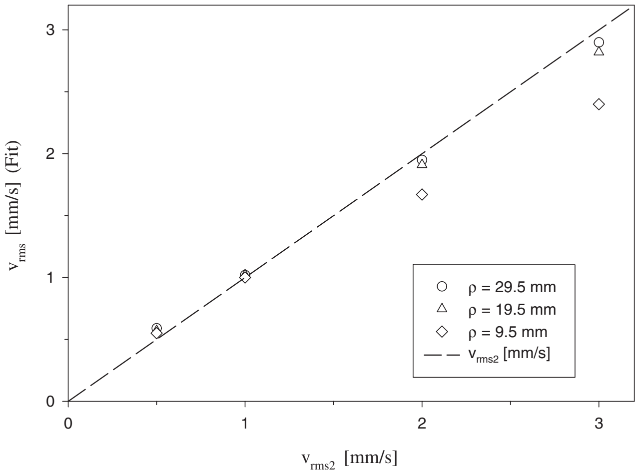

Figure 8. Estimated $v_{\rm rms}$ determined by nonlinear regression of the homogeneous to the two-layered solution of the correlation diffusion equation. The optical properties of the two-layered medium are $\mu'_{s1} = 1.2$ mm^{-1}, $\mu_{a1} = 0.01$ mm^{-1}, $\mu'_{s2} = 0.5$ mm^{-1}, $\mu_{a1} = 0.02$ mm^{-1} and $n = 1.4$. The root-mean-square velocities of the first and the second layers are $v_{\rm rms1} = 1$ mm s^{-1} and $v_{\rm rms2} = 0.5$–3 mm s^{-1}, respectively, and $P_{b1} = 0.02$, $P_{b2} = 0.1$. The thickness of the first layer is $l = 2$ mm.

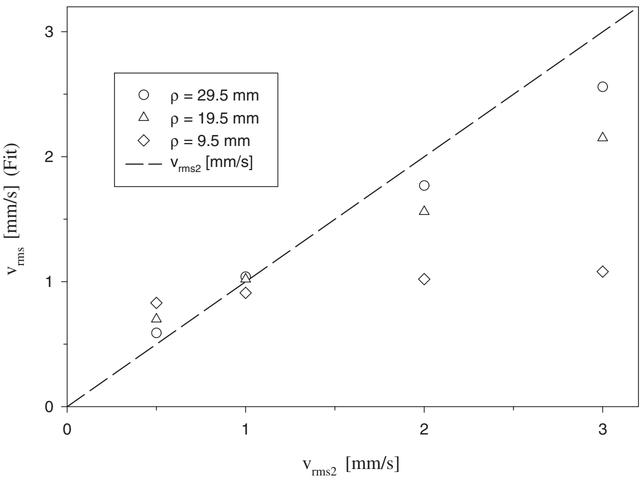

Figure 9. Estimated $v_{\rm rms}$ determined by nonlinear regression of the homogeneous to the two-layered solution of the correlation diffusion equation. The optical properties of the two-layered medium are $\mu'_{s1} = 1.2$ mm^{-1}, $\mu_{a1} = 0.01$ mm^{-1}, $\mu'_{s2} = 0.5$ mm^{-1}, $\mu_{a1} = 0.02$ mm^{-1} and $n = 1.4$. The root-mean-square velocities of the first and the second layers are $v_{\rm rms1} = 1$ mm s^{-1} and $v_{\rm rms2} = 0.5$–3 mm s^{-1}, respectively, and $P_{b1} = 0.02$, $P_{b2} = 0.1$. The thickness of the first layer is $l = 5$ mm.

4. Discussion

Laser Doppler apparatus for measurement of the blood microcirculation of tissue deliver perfusion values in relative units only, which are normally derived from the first moment of the laser Doppler spectra. In contrast, laser Doppler measurements simultaneously performed

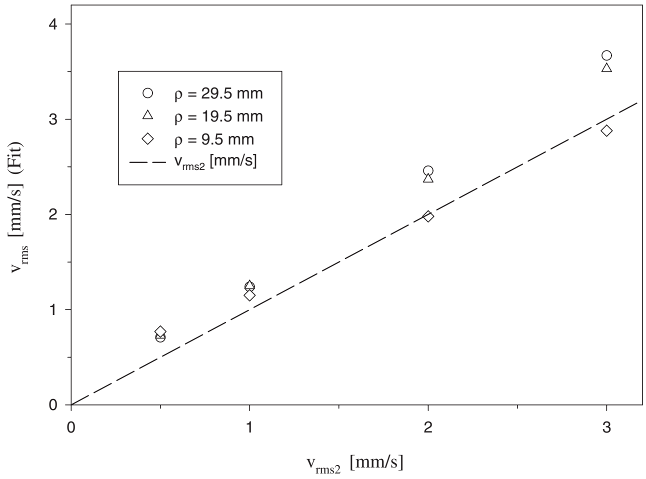

Figure 10. Estimated v_{rms} determined by nonlinear regression of the homogeneous to the two-layered solution of the correlation diffusion equation. A reduced scattering coefficient of the homogeneous turbid medium of $\mu_s' = 0.5\ \mathrm{mm}^{-1}$ ($\mu_a = 0.02\ \mathrm{mm}^{-1}$) was assumed. The optical properties of the two-layered medium are $\mu_{s1}' = 1.2\ \mathrm{mm}^{-1}$, $\mu_{a1} = 0.01\ \mathrm{mm}^{-1}$, $\mu_{s2}' = 0.7\ \mathrm{mm}^{-1}$, $\mu_{a1} = 0.02\ \mathrm{mm}^{-1}$ and $n = 1.4$. The root-mean-square velocities of the first and the second layers are $v_{\mathrm{rms1}} = 1\ \mathrm{mm\ s}^{-1}$ and $v_{\mathrm{rms2}} = 0.5$–$3\ \mathrm{mm\ s}^{-1}$, respectively, and $P_{b1} = 0.02$, $P_{b2} = 0.1$ are used. The thickness of the first layer is $l = 2$ mm.

with measurements of the optical properties of tissues enable absolute values of the blood concentration and velocity to be obtained. The optical properties can be readily determined provided that the tissue involved can be regarded as semi-infinite and homogeneous and the distance between the source and detector is large ($\rho \gg \mu_s'$). However, as is indicated by the correlation diffusion equation for random flow (see equation (4) using $\langle \Delta r^2(\tau) \rangle = \langle v^2 \rangle \tau^2$) the concentration (more exactly P_b) and the mean square velocity of blood cannot be separately determined for large ρ and large P_b values. Therefore, under these conditions it is necessary to determine the blood concentration independently in order to obtain the blood velocity. The blood concentration can be derived from the measurements of the optical properties at different wavelengths and from comparison of the obtained absorption coefficients with the known molar absorption coefficients of blood. Knowing the blood concentration and the optical properties of the semi-infinite homogeneous turbid medium at the wavelength used for the laser Doppler measurements, the v_{rms} velocity can be easily found by comparing the calculated and the measured spectra. In this case it is even sufficient to compare only the first moments of the spectra.

In this study we also investigated the determination of the blood velocity for semi-infinite media that are not homogeneous. In particular, the possibility of obtaining the root-mean-square velocity of blood in muscles of the extremities was examined. It was shown, by nonlinear regression of laser Doppler spectra calculated using the solution for a semi-infinite homogeneous turbid medium to data obtained from a two-layered turbid medium representing the muscle and the layers above the muscle, that the differences between the derived v_{rms} and v_{rms} of the muscle layer are less than 20% for the cases investigated if the optical coefficients of the muscle (hence also c_b) are known and the distances ρ are large. It has been shown in the literature that the absorption coefficient of muscle can be accurately obtained from near-infrared spectroscopy measurements in the frequency or time domains if the thickness of the layers above the muscle is less than about 4 mm (Franceschini *et al* 1998, Kienle and

Glanzmann 1999), whereas the errors in determining the reduced scattering coefficients are larger (Kienle and Glanzmann 1999). In this study the derivation of $v_{\rm rms}$ was also investigated for a typical error in the determination of μ_s' for muscle. It was found that, in general, this error increases the differences between the derived $v_{\rm rms}$ and the $v_{\rm rms}$ of the muscle. In order to improve the determination of the reduced scattering coefficient, and also of the absorption coefficient of muscle, a solution of the diffusion equation for two layers can be used (Kienle *et al* 1998, Alexandrakis *et al* 1998, Tualle *et al* 2000).

For the blood layer we used $P_{b2} = 0.1$. This is close to the average value we found for blood concentration in muscle of the extremities measured by near-infrared spectroscopy (Hamaoka *et al* 2000, Cubeddu *et al* 1999) and the ratio of the reduced scattering coefficient of muscle tissue and whole blood (Cubeddu *et al* 1999, Fantini *et al* 1995, Kienle and Glanzmann 1999, Roggan 1997, Kienle 1994). For the layers above the muscle layer (skin and subcutaneous fat) we used $P_{b1} = 0.02$. This value is a worst case assumption as the blood concentration is reported to be of the order of 1 vol.% in skin (van Gemert *et al* 1997, Verkruysse *et al* 1993) and the blood concentration in the subcutaneous fat layer is less than in skin. The smaller P_{b1}, the more accurate the $v_{\rm rms}$ value for the muscle layer which can be derived, because the contribution of the blood above the muscle to the laser Doppler spectra decreases.

A solution of the correlation diffusion equation was used to investigate the possibility of determining the blood velocity in muscle. For the calculation of the laser Doppler spectra of the two-layered media, which served as 'experimental data', we also used a solution of the correlation diffusion equation. It was shown that the solutions of the correlation diffusion equation for semi-infinite homogeneous and two-layered turbid media are only good approximations to Monte Carlo simulations if the re-emitted photons have experienced a large number of interactions with the moving particles. To avoid errors due to the correlation diffusion approximation used in the nonlinear regression to fit experimental data (for example, if $\rho \gg (\mu_s')^{-1}$ is not fulfilled), the Monte Carlo method can be used in the nonlinear regression. It is sufficient to perform one Monte Carlo simulation to obtain a laser Doppler spectrum for a homogeneous turbid medium using the known optical properties of the muscle layer. Then, for the nonlinear regression algorithm this spectrum can be scaled to obtain the spectra for different root-mean-square velocities of the flowing scatterers in the turbid medium, because the frequency shift is proportional to the velocity of the moving particles (see equation (2)). To further accelerate the nonlinear regression, a hybrid model, which uses the Monte Carlo method for small distances and diffusion theory for large distances, can be used (Wang and Jacques 1993, Alexandrakis *et al* 2000).

For semi-infinite homogeneous media it was shown that the laser Doppler spectra do not depend on the anisotropy factor of the turbid medium provided that the number of interactions with the moving scatterers is large, which is the case for blood concentrations found in muscles and $\rho \geqslant 20$ mm. We also showed that under these conditions the influence of the velocity distribution of the moving particles is negligible.

Additionally, it was demonstrated that laser Doppler spectra can be obtained more quickly from Monte Carlo simulations that score the distribution of the modulus of the wavevector, which is used to calculate the autocorrelation function, than from Monte Carlo simulations that directly calculate the laser Doppler spectra in the frequency domain. The disadvantage of the first is that it is only valid for a Gaussian velocity distribution. However, for the blood concentration found in muscle and the large distances investigated in this study the velocity distribution does not strongly influence the spectra.

Future work towards *in vivo* measurements include the comparison of experimental laser Doppler spectra measured on layered phantoms with the theoretical data presented in this study. (The comparison using cylindrical models has been already successfully performed by

Kienle *et al* (1996).) Also, using measurements or simulations with a three-layered model, it has to be ascertained if the skin and the fat layers can be considered as one layer. We expect that regarding the skin and fat layers as one layer does not significantly change the results obtained because both layers have a small blood concentration (more exactly P_b) compared with the muscle layer.

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