Determination of the scattering coefficient and the anisotropy factor from laser Doppler spectra of liquids including blood

Alwin Kienle, Michael S. Patterson, Lutz Ott, and Rudolf Steiner

Laser Doppler measurements were performed on scattering liquids flowing through a highly scattering static medium to determine the scattering coefficient and the anisotropy factor of the liquids. Monte Carlo simulations of light propagation in the static and moving media were used to calculate the Doppler spectra for suspensions of polystyrene spheres in water, and these spectra were in excellent agreement with experimental results. A faster Monte Carlo code was developed so that nonlinear regressions to the measured laser Doppler spectra could be used to determine the anisotropy factor of other liquids. This approach was used to deduce the scattering properties of Intralipid and blood at $\lambda = 820$ nm. It was found that the anisotropy factor of blood is well described by Mie theory in contradiction to results reported in the literature that were obtained by goniometric measurement of the phase function.
Key words: Laser Doppler, Monte Carlo, anisotropy factor, blood.

1. Introduction

Knowledge of the optical properties of biological tissues is important in the applications of lasers in medicine. Many studies have been conducted to investigate the absorption and scattering coefficients of soft and hard tissues.[1] In principle, these methods can also be used to derive the scattering and absorption characteristics of liquids, such as blood.

In contrast to soft or hard tissue, the optical coefficients of scattering fluids may be deduced from the properties of their scattering particles if the volume fraction of particles is low enough that each acts independently. Frequently, Mie theory is used to compute the absorption coefficient μ_a, the scattering coefficient μ_s, and the phase function $p(\theta)$, where θ is the scattering angle. For some purposes, such as modeling light propagation in multiple-scattering media, $p(\theta)$ can be represented by the anisotropy factor g, which equals the average cosine of the scattering angle. Several fluids, particularly suspensions of polystyrene spheres and Intralipid, a lipid emulsion (Abbott Laboratories, Montreal, Quebec), have been well characterized and used as tissue-simulating phantoms.

However, despite its importance, the optical coefficients of diluted blood have not been investigated to the extent that tissues have. The applicability of Mie theory is not obvious because the main scatterers in blood (the erythrocytes) are biconcave disks and not spheres, as supposed in Mie theory. Steinke and Shepherd[2] measured the collimated transmittance of dilute blood suspensions to obtain the scattering coefficient μ_s, and they derived the anisotropy factor g by making angular scattering measurements in a goniometer. They found that the anisotropy factor calculated with Mie theory was greater than that obtained experimentally, although μ_s was well described by Mie theory. However, blood cells scatter light predominantly in the forward direction, and it is difficult to measure the correct g value using a goniometer.

In this study we investigate a new method to measure the scattering coefficient and the anisotropy factor of scattering liquids. We use a laser Doppler apparatus that is normally applied in medicine to the measurement of blood flow in tissue.[3] The liquid flows through a highly scattering static medium. A Doppler shift occurs at each scattering

A. Kienle, L. Ott, and R. Steiner are with the Institut für Laser-Technologien in der Medizinune Messtechnik, Helmholtzstrasse 12, 89081 Ulm, Germany. M. S. Patterson is with the Department of Medical Physics, Hamilton Regional Cancer Center, 699 Concession Street, Hamilton, Ontario, L8V 5C2 Canada; A. Kienle is also with the Department of Medical Physics, Hamilton Regional Cancer Centre.

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of the incident light by the moving particles of the liquid. As a result of the scattering in the static medium, light is incident upon the moving liquid from all directions, and many different Doppler shifts occur, producing a Doppler spectrum. Light may also undergo multiple Doppler scattering before it is detected. The Doppler frequency shift at each scattering interaction is proportional to $\sin(\theta/2)$. For high g values a small change in g is related to a relatively large change in the average scattering angle, so laser Doppler spectra are sensitive to small changes of g.

The Monte Carlo method was used to simulate light propagation in the static and flowing media and hence to calculate the laser Doppler spectrum. We show that the scattering coefficient of liquids can readily be derived from the integration of the Doppler spectrum provided that the absorption coefficient μ_a of the liquid is much smaller than the scattering coefficient. Even if μ_a is significant, the determination of μ_s is also possible, but a separate measurement of $\mu_a + \mu_s$ is necessary. The anisotropy factor was deduced by the application of an iterative, least-squares nonlinear-regression method to fit the measured Doppler spectra.[4] For reducing the long computation time a simpler and faster Monte Carlo simulation code for the calculation of the laser-Doppler spectrum was developed. The experimental setup was tested by comparison of the laser Doppler measurements of polystyrene spheres with Monte Carlo simulations. Finally, the scattering properties of dilute Intralipid–20% (Liposyn, 20% solids) and blood were determined at $\lambda = 820$ nm.

2. Experimental Setup

In Fig. 1 a schematic drawing of the experimental setup is shown. Measurements of the laser Doppler spectra were made with a modified commercial apparatus (DWL Elektronische Systeme GmbH, Sipplingen, Germany). Light from a laser diode (4 mW) emitting at 820 nm was coupled into an optical fiber (light fiber) with a core diameter of 600 μm. The fiber was fixed in a probe placed perpendicular to the surface of the highly scattering medium, Teflon (polytetrafluorethylene). At each side of the fiber a photodiode with a light-sensitive area of 0.25 mm^2 was located at a distance of 1.55 mm from the center of the fiber. The photons, which are scattered in the turbid media and reemitted from the Teflon slab, were detected by the use of the photodiodes. In the detectors a beat signal is produced from the interference between the frequency-shifted photons and those which are only elastically scattered. The signals from these two detectors are applied to a difference amplifier (D) to improve the signal-to-noise ratio.[5] From the output signal of the difference amplifier the laser-Doppler spectrum (power spectrum) $S(\nu)$ can be calculated by PC with the Fourier transformation. Because the signal is high-pass filtered, Doppler frequencies below 100 Hz cannot be measured with our device.

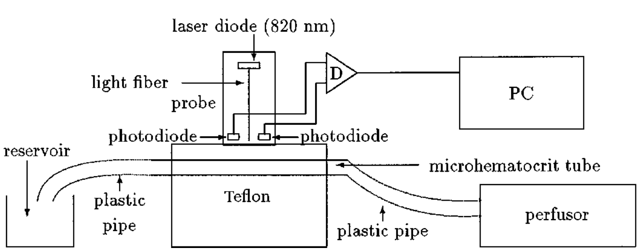

Fig. 1. Schematic diagram of the experimental setup for the measurement of laser Doppler spectra. D, differential amplifier.

A circular hole was drilled parallel to the surface of the Teflon block at a depth of 1 mm, and a glass microhematocrit tube with an inner diameter of 1.2 mm and a wall thickness of 0.2 mm was inserted into the block. A perfusor normally used for medical applications pumped the scattering liquid under investigation, by means of a plastic pipe, to the microhematocrit tube. Another plastic pipe was used to drain the liquid at the other end of the microhematocrit tube into a reservoir. For the experiments in this study a flow rate was used that resulted in an average velocity of 2.50 ± 0.15 mm/s in the microhematocrit tube. With the assumption of a parabolic velocity distribution over the cross section of the tube, the maximum velocity was 5.0 ± 0.3 mm/s at the center of the tube. The uncertainty in the velocity results from the measurement of the diameter of the microhematocrit tube and the liquid volume pumped per second by the perfusor.

For the theoretical calculation of the laser Doppler spectra the optical coefficients of the static medium (Teflon) have to be known. We used an integrating sphere to measure the total diffuse transmission and reflection of the Teflon slabs. A xenon high-pressure lamp served as the light source. The reduced scattering coefficient $\mu_s' = \mu_s(1 - g)$ and the absorption coefficient μ_a were deduced from these measurements with an inverse Monte Carlo technique similar to that applied by Roggan *et al.*[6] Three slabs—two of which were 2 mm thick and one that was 3 mm thick—were measured, and we computed values of $\mu_s = 24 \pm 1$ mm^{-1} and $\mu_a = 0.001 \pm 0.001$ mm^{-1} at $\lambda = 820$ nm, assuming $g = 0.9$. For this highly scattering medium and for the geometry in this study, an accurate estimate of the anisotropy factor was not required for the calculation of light propagation. The absorption coefficient could be determined only approximately, because the mean free path for absorption was much greater than the thickness of the sample slabs. However, we checked that this uncertainty did not affect the results of this study by calculating the laser Doppler spectra for different absorption coefficients of Teflon. For the refractive index of Teflon $n = 1.35$ was used.[7] The fiber of the optical probe was not placed directly onto the Teflon block, nor were the photodiodes optically isolated from the incident light of the fiber in the optical probe, to ensure that the detected amount of non-Doppler-shifted light was much greater than that of the Doppler-shifted light. Therefore, heterodyne detection can be assumed in the analysis.[8,9] We also found that, for this probe, the measured laser Doppler spectra did not change when the probe was rotated around the axis of the delivering fiber. Thus, in the Monte Carlo simulations it was not necessary to score two-dimensional information about the location of each photon escape but only the distance from the source. For the blood measurements whole blood was drawn directly from a vein of a male volunteer into a heparinized tube. The blood was then shaken in air to ensure oxygenation. It was diluted with isotonic saline and used for experiments within 2 h of drawing.

3. Theory

A. Monte Carlo Method for the Simulation of Laser Doppler Spectra

Laser Doppler flowmetry is based on the interference of frequency-shifted and non-frequency-shifted light at the detector that generates intensity modulation at the Doppler frequency Δf. For nonrelativistic particle velocities Δf can be calculated from

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

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

where f_0 is the frequency of the incident photon, $\mathbf{v}$ is the velocity of the moving particle, c is the velocity of light in the medium, and $\Delta\mathbf{k}$ is the difference of the scattered and incident wave vectors.[8] The angle between $\Delta\mathbf{k}$ and the velocity of the scattering particle is termed δ. For investigations of blood flow in tissue the dependence of the frequency shift on the velocity or concentration of the scattering particles is used,[5,10] whereas in this study we used the frequency-shift dependence on the scattering angle and on the concentration. For deducing the scattering coefficient and the anisotropy factor from the laser Doppler spectrum, a theoretical model for light propagation in the static and flowing media must be applied.

Monte Carlo simulations are best suited for the problem of light propagation in static and flowing media and have been used in related studies, such as laser Doppler flowmetry.[8,9,11,12] Figure 2 shows a diagram of a cross section of the geometry for the Monte Carlo simulations and the experiments. Photons were perpendicularly incident on the highly scattering Teflon slab, which is 13 mm thick. The lateral dimensions of the slab were assumed to be infinite in the simulations. If not explicitly stated, we assumed a parabolic velocity distribution with a maximum velocity of $v_m = 5$ mm/s in the center of the hole.

Calculating light propagation in turbid media with the Monte Carlo method is thoroughly described in the literature.[13,14] The optical coefficients-for the static medium were $\mu_s = 24$ mm^{-1}, $\mu_a = 0.001$ mm^{-1}, $g = 0.9$, and $n = 1.35$ (see Section 2). In the static medium we applied the Henyey–Greenstein phase function. If the photons penetrate into the moving medium, the optical parameters of that medium have to be used. Because the refractive index n of the static medium ($n = 1.35$) was close to that of the liquids ($n = 1.33$), Fresnel reflection at the boundary was ignored, as was the influence of the transparent microhematocrit tube. At each scattering point in the moving medium the Doppler frequency shift has to be calculated with Eq. (2). For the Monte Carlo simulations in this section (as opposed to the Monte Carlo code of section 3.C) it is more convenient to use the form[11]

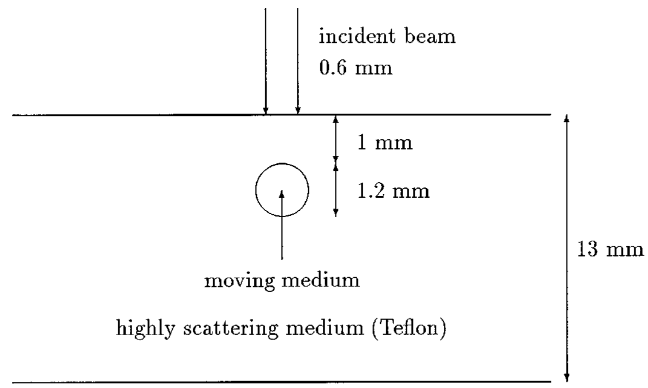

Fig. 2. Representation of a cross section of the geometry used in the Monte Carlo simulations. The lateral dimensions are assumed to be infinite.

$$\Delta f = \frac{f_0}{c} \mathbf{v}(\mathbf{e}_2 - \mathbf{e}_1), \tag{3}$$

where $\mathbf{e}_2$ and $\mathbf{e}_1$ are the unit vectors in the scattered and incident directions, respectively. If a photon is scattered more than once in the moving medium, all frequency shifts have to be summed to get the total frequency shift of the simulated photon. All remitted photons, independent of the emission angle, are scored because the highly scattering static medium removes any dependence of the Doppler spectrum on the emission angle.[9] The number of photons remitted from the surface of the static medium was scored as a function of the distance from the incident beam and the Doppler shift. The Doppler spectrum $S(\nu)$ was calculated with[9]

$$S(\nu) = S(i\Delta\nu) = C N_0 N_i. \tag{4}$$

In Eq. (4) N_i represents the number of photons in the frequency interval between $(i - 1/2)\Delta\nu$ and $(i + 1/2)\Delta\nu$, where $\Delta\nu$ is the width of the elements of the frequency array in the Monte Carlo simulations. N_0 is the number of all photons remitted without a frequency shift, and the quantity C represents properties of the detector and the degree of coherence of different frequency intervals. In this study, it is assumed that C is constant and that N_0 is much greater than N_i (heterodyne detection).[9]

For the investigation of the dependence of Doppler spectra on the lateral positions of the detectors, laser Doppler spectra were calculated for different distance ranges. Simulations were performed for polystyrene-sphere suspensions with coefficients of $\mu_s = 0.25\ \mathrm{mm}^{-1}$ and $\mu_a = 0.002\ \mathrm{mm}^{-1}$. Phase functions were calculated with Mie theory and a refractive index of $n = 1.59$ for polystyrene. The result was that all simulated curves for the different distance ranges show approximately the same form. The reason for this similarity is that the distribution of photon directions incident on the moving medium, which is ≈2.4 transport mean free paths below the Teflon surface, is isotropic because of the highly scattering static medium. However, the probability that a Doppler-shifted photon is remitted at larger lateral distances from the incident beam decreases because of the greater path in the scattering medium, so the absolute values of the curves are different. If only relative values of the Doppler spectrum $S(\nu)$ are considered (as in this study), it is possible to use all remitted photons regardless of the lateral distance of the reemission relative to the incident beam.

However, despite this variance reduction, these simulations require long computation times to achieve useful precision. Deducing the anisotropy factor from the laser Doppler spectra requires an iterative, nonlinear-regression procedure. This process leads to prohibitively long computation times if the Monte Carlo method described above is used. Therefore, we have investigated a simpler model, described in Section 3.C, that reduces the computation time by several orders of magnitude.

B. Determination of the Scattering Coefficient

To investigate how the scattering coefficient μ_s of the liquid can be deduced from the laser Doppler spectrum, we scored the number of scattering events in the moving medium for each photon that was remitted with the Monte Carlo program. Figure 3 shows the probability distribution of the number of scattering events with the geometry from Fig. 2 for polystyrene spheres with diameters of $d = 300, 519, 806$ nm and for blood as the flowing media. For the moving media phase functions were calculated from Mie theory. Red blood cells were approximated as spheres with a diameter of $d = 5.58\ \mu\mathrm{m}$ (Ref. 2) and a refractive index of $n_\tau = 1.036$ relative to the plasma.[15] Scattering and absorption coefficients of $\mu_s = 0.25\ \mathrm{mm}^{-1}$ and $\mu_a = 0.002\ \mathrm{mm}^{-1}$, respectively, were used for all moving media. The absorption of the polystyrene-sphere suspensions is mainly due to water. Thus, we used a value of $\mu_a = 0.002\ \mathrm{mm}^{-1}$, which approximately equals the μ_a of water at 820 nm.[16] The absorption coefficient of blood is greater than a factor of 100 smaller than the scattering coefficient at 820 nm.[15] Therefore, for diluted blood with $\mu_s = 0.25\ \mathrm{mm}^{-1}$, the absorption coefficient (including the contribution of water) has an approximate value of $\mu_a = 0.004\ \mathrm{mm}^{-1}$. The same simulations as were shown in Fig. 3 were also made for this absorption

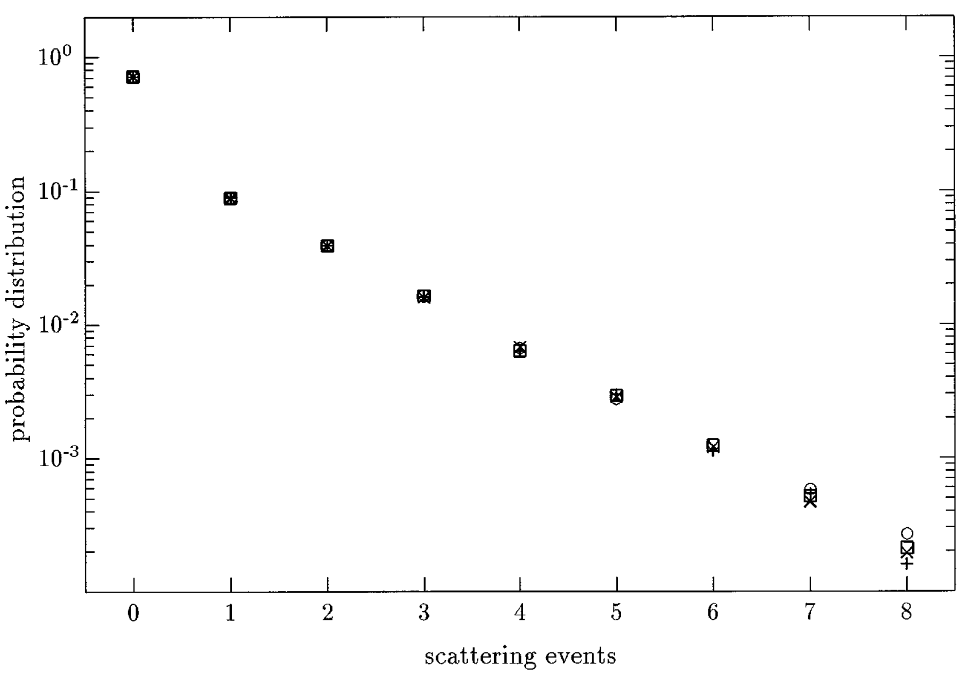

Fig. 3. Plot of the probability distribution of the number of Doppler-scattering events for a reemitted photon. The moving media were polystyrene spheres with diameters of $d = 300$ nm (pluses), $d = 519$ nm (crosses), $d = 806$ nm (open squares) and blood (open circles). The velocity distribution of the moving medium was assumed to be parabolic, with a maximum velocity of $v_m = 5$ mm/s. The optical coefficients of the moving fluids had values of $\mu_s = 0.25\ \mathrm{mm}^{-1}$ and $\mu_a = 0.002\ \mathrm{mm}^{-1}$. These data were generated with 156,000 photon histories in the Monte Carlo simulation.

coefficient, and no difference was found in the probability distributions.

Figure 3 shows that the probability distribution of the number of scattering events is independent of the phase function of the moving medium for these μ_a and μ_s values. This independence is because the mean free path of the photons in the liquid is much greater than the diameter of the cylinder in which the liquid flows. Therefore, scattering locations are approximately uniformly distributed over the whole cross section of the moving medium. This uniformity of distribution means that the total number of Doppler-shifted photons reemitted at the surface depends only on the scattering and absorption coefficients of the moving medium.

If the absorption coefficient is much smaller than the scattering coefficient, the scattering coefficient of the moving medium can be derived from a change in the concentration of the liquid until the integrated Doppler spectrum equals that of the calibration measurements. For the calibration measurements we used polystyrene spheres with different diameters and at concentrations that resulted in the following values for the optical coefficients: $\mu_s = 0.25\ \text{mm}^{-1}$ and $\mu_a = 0.002\ \text{mm}^{-1}$. If the absorption coefficient is not much smaller than the scattering coefficient, the determination of μ_s is still possible (see Section 5).

C. Determination of the Anisotropy Factor

If the scattering coefficient of the liquid is known, the anisotropy factor g can be determined from fitting the theoretical laser Doppler spectrum calculated with the Monte Carlo method to the experimental spectra. However, to be practical, the long calculation time for the Monte Carlo method has to be reduced.

In the Monte Carlo simulations described in Section 3.A. nearly all the computation time was spent to track photons in the static turbid medium, and only a small amount was needed for simulation of the scattering processes in the moving medium. Thus the simulation time could be drastically reduced if the effects of the static medium were modeled in a simpler way. To explore this, we used the full Monte Carlo simulations and scored the distributions of δ and of the velocity of the moving medium for all scattering events in the liquid. For all simulations shown in Fig. 3 it was found that the angle distribution was isotropic and that the velocity distribution was uniform from $v = 0$ mm/s to $v = v_m$. The former finding is a result of the highly scattering static medium, whereas the latter finding is a result of the fact that, for a parabolic velocity distribution within a circular cross section, all velocities are equally probable.[17]

With this knowledge, which implies that the velocity of the scatterers is not correlated with the angle, δ, it is possible to design a simpler method for the calculation of the Doppler spectra. It is necessary to make only one simulation with the normal Monte Carlo code and then to score the probability distribution of the number of scattering events in the moving medium for fixed values of μ_s and μ_a for the moving medium (in this study we used values of $\mu_s = 0.25\ \text{mm}^{-1}$ and $\mu_a = 0.002\ \text{mm}^{-1}$). Then, with this information and the uniform distributions of the velocities and incident angles, the Doppler spectrum can be calculated with Eq. (2) and simple Monte Carlo algorithms. For the calculation of Δf for a single photon, first the number of scattering events is sampled from the above-mentioned probability distribution (see Fig. 3) with the usual Monte Carlo procedure. Then, for each event, the scattering angle is sampled from the appropriate phase function. The frequency shift for a scattering event is calculated with Eq. (2), where v and δ are sampled from a uniform velocity distribution that ranges from $v = 0$ mm/s to $v = v_m$ and from an isotropic angle distribution, respectively. This procedure is repeated for all scattering events of the single photon to deduce its total frequency shift. Finally, Δf is calculated for a great number of photons to estimate the laser Doppler spectrum; typically, 100,000 photons were used. For the geometry and the optical coefficients used in this study, the laser Doppler spectra can be calculated more than 1000 times faster than with the full Monte Carlo simulation described above.

In the iterative nonlinear regression for determining the anisotropy factor the analytic form of the phase function is not generally known. Therefore, we used the one-parameter Henyey–Greenstein function

$$p(\theta) = \frac{1}{4\pi} \frac{1 - g^2}{[1 + g^2 - 2g\cos(\theta)]^{3/2}}. \tag{5}$$

In Eq. (5) the parameter g equals the anisotropy factor. We also investigated the two-parameter phase function of Reynolds and McCormick[18]:

$$f(\theta) = K[1 + g'^2 - 2g'\cos(\theta)]^{-(\alpha+1)},$$
$$K = \pi^{-1}\alpha g'(1 - g'^2)^{2\alpha}[(1 + g')^{2\alpha} - (1 - g')^{2\alpha}]^{-1}. \tag{6}$$

For $\alpha = 1/2$, Eqs. (6) reduce to the one-parameter Henyey–Greenstein function. The anisotropy factor g can be calculated from Eqs. (6) by the use of Eqs. (7):

$$g = [2g'\alpha L - (1 + g'^2)]/[2g'(\alpha - 1)],$$
$$L = [(1 + g')^{2\alpha} + (1 - g')^{2\alpha}]/[(1 + g')^{2\alpha} - (1 - g')^{2\alpha}]. \tag{7}$$

In Section 4.B. the Doppler spectra computed with both phase functions are used in a nonlinear-regression routine to fit spectra calculated with Mie theory. The logarithmic values of the spectra are fitted, and the same weight factors are used for all experimental data.

4. Results

A. Comparison of Experimental Doppler Spectra and Monte Carlo Simulations

To compare the experimental and theoretical Doppler spectra, we conducted measurements with polystyrene microspheres ($d = 519$ nm and $d = 806$ nm) and calculated $S(\nu)$ with the full Monte Carlo code, which includes light propagation within the Teflon block. As shown in Fig. 4, the slope of the Doppler spectrum $S(\nu)$ versus the frequency for polystyrene spheres with $d = 519$ nm is not as steep as that for spheres with $d = 806$ nm because the average scattering angle, hence the Doppler shift Δf, is greater [see Eq. (1)]. The experimental curves have been scaled by the same factor to provide the best match to the theoretical results. Good agreement between the shapes of the theoretical and experimental curves was obtained. In the Monte Carlo simulations we used a maximum velocity of $v_m = 5$ mm/s in the center of the flowing liquid because v_m was determined as $v_m = 5.0 \pm 0.3$ mm/s (see Section 2). Figure 4 shows that the slopes of the theoretical curves are somewhat smaller than those of the experimental curves. The curves can be better matched if a value of $v_m \approx 4.75$ mm/s is used; and this value was applied in Section 4.C for the determination of the optical coefficients of Intralipid and blood.

Figure 5 shows a comparison of $S(\nu)$ calculated with the full Monte Carlo code, which explicitly includes the static medium, and the simpler Monte Carlo code described above (Section 3.C.). The comparison reveals that the full Monte Carlo simulation (dashed curve) and the simpler code (solid curve) produce approximately the same results, although a small difference can be seen at high frequencies. We postulated that this difference was due to correlations between δ and the velocity of the particles at the scattering locations of photons that scatter more than once before they leave the flowing liquid. To check this statement we made simulations with the full Monte Carlo program but chose the velocity of the scattering particles from a uniform distribution between zero and the maximum velocity, independent of the location of the scattering events. Thus, the correlation should be suppressed. Figure 5 shows that this verifying simulation (long-dashed curve) yields a laser Doppler spectrum equivalent to the result of the simpler model. Because this slight difference does not influence the determination of the optical properties to a great extent, especially if the anisotropy factor is high, we concluded that the simpler model could be used to calculate the laser Doppler spectra. The simpler Monte Carlo method can be applied to demonstrate the dependence of a laser Doppler spectrum on g. For the g-dependence calculations the Henyey–Greenstein function was used to represent the phase function of the moving liquid. Figure 6 shows the values of $S(\nu)$ for anisotropy factors of $g = 0$, 0.5, 0.8, 0.9, 0.95, 0.98, 0.99, 0.995. It can be seen that a small change in g makes a great difference in $S(\nu)$ if g is close to one. Therefore, it should be possible to determine accurately the anisotropy factors of media that are highly forward scattering, such as blood.

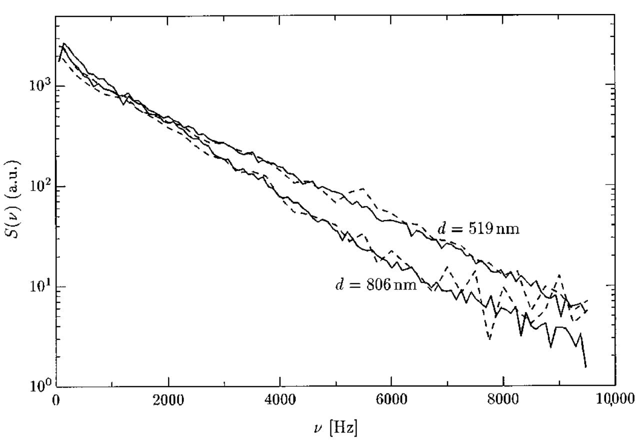

Fig. 4. Comparison of experimental (solid curves) and calculated (dashed curves) laser Doppler spectra for polystyrene spheres with diameters of $d = 519$ nm and $d = 806$ nm flowing through the Teflon block. The optical coefficients of the moving fluids had values of $\mu_s = 0.25$ mm^{-1} and $\mu_a = 0.002$ mm^{-1}.

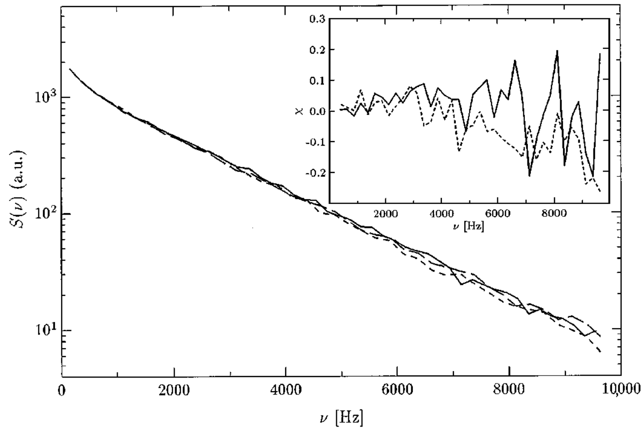

Fig. 5. Comparison of laser Doppler spectra calculated with the full Monte Carlo program (dashed curve) and with the simpler code (solid curve). With the use of the full Monte Carlo code, $S(\nu)$ was also calculated under the assumption that there was no correlation between the velocities of the scatterers and δ (long-dashed curve). A liquid medium consisting of polystyrene spheres with diameters of $d = 519$ nm, $\mu_s = 0.25$ mm^{-1}, and $\mu_a = 0.002$ mm^{-1} was simulated. In the inset the normalized residuals χ of these curves are shown. The dashed curve represents χ for the curves calculated with the full Monte Carlo program, with and without correlations, and the solid curve represents χ for the curves calculated with the full Monte Carlo program, without correlation, and the simpler Monte Carlo code.

B. Investigation of Different Phase Functions

If the scattering coefficient of the investigated liquid is determined with the method explained above (Section 4.A.) or is known *a priori,* the concentration of the liquid can be chosen to produce a reference value of μ_s; in our case, the value chosen was $\mu_s = 0.25$ mm^{-1}. The anisotropy factor can be deter-

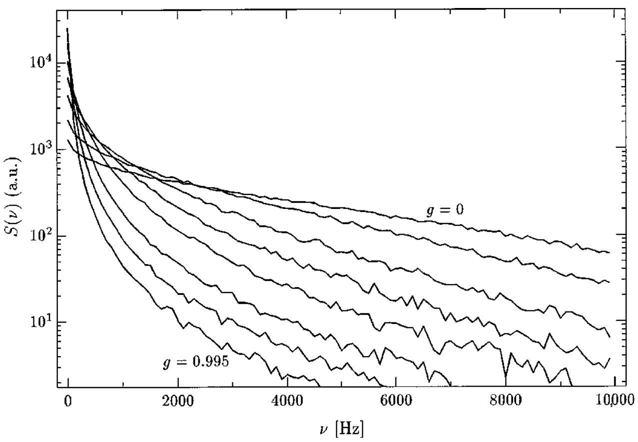

Fig. 6. Plots of $S(\nu)$ for moving liquids that have the following anisotropy factors of the Henyey–Greenstein phase function: $g = 0, 0.5, 0.8, 0.9, 0.95, 0.98, 0.99, 0.995$. The curves for $g = 0$ and $g = 0.995$ are identified in the figure. The slopes of the others change continuously with g. The scattering and absorption coefficients are $\mu_s = 0.25\ \text{mm}^{-1}$ and $\mu_a = 0.002\ \text{mm}^{-1}$, respectively.

mined with a nonlinear regression to fit the experimental Doppler spectrum. To investigate whether it is possible to use the Henyey–Greenstein function in the iterative Monte Carlo approach, we fitted $S(\nu)$ calculated with this phase function to the theoretical laser Doppler spectra calculated for polystyrene spheres of $d = 519$ nm and $d = 806$ nm with phase functions computed with Mie theory. In Fig. 7 it can be seen that the best-fit spectrum generated with the Henyey–Greenstein phase function does not correspond to the true Doppler spectrum. The reason for this is that the Henyey–Greenstein function is not a good approximation to the phase functions calculated with Mie theory. The differences

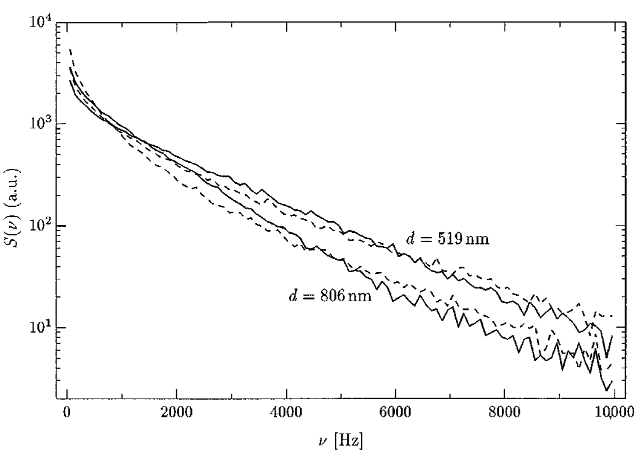

Fig. 7. Plots of the laser Doppler spectra $S(\nu)$ for polystyrene-sphere suspensions with diameters of $d = 519, 806$ nm as the moving liquid. $S(\nu)$ was determined with Mie-theory phase functions (solid curves) and a nonlinear regression to these curves that applied the Henyey–Greenstein function (dashed curves). The scattering and absorption coefficients of the polystyrene-sphere suspensions are $\mu_s = 0.25\ \text{mm}^{-1}$ and $\mu_a = 0.002\ \text{mm}^{-1}$, respectively.

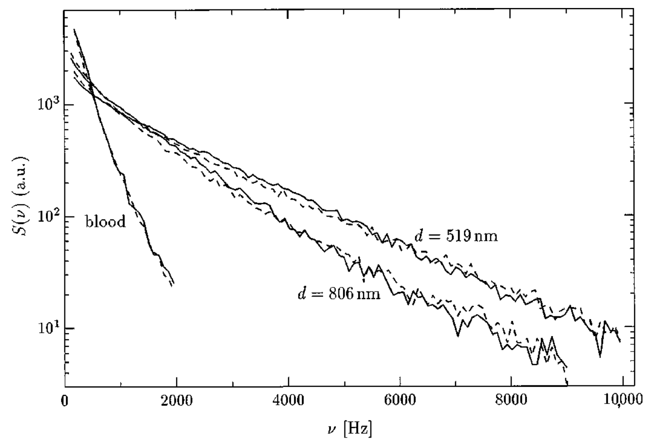

Fig. 8. Plots of the laser Doppler spectra $S(\nu)$ for polystyrene-sphere suspensions with diameters of $d = 519, 806$ nm and for blood (assume that the red blood cells are spheres with $d = 5.58$ µm) as the moving liquids. The $S(\nu)$ were determined with Mie-theory phase functions (solid curves) and a nonlinear regression to these curves that applied the Reynolds–McCormick phase function (dashed curves). The scattering and absorption coefficients of the flowing liquids are $\mu_s = 0.25\ \text{mm}^{-1}$ and $\mu_a = 0.002\ \text{mm}^{-1}$, respectively.

between these types of phase functions is important for light propagation in turbid media if the average number of scattering events is small, as it was in our experiment. Using the Reynolds–McCormick phase function, which provides a better approximation to Mie theory,[18] yields the superior results shown in Fig. 8, in which the spectra for blood are also shown.

Table 1 compares the anisotropy factors g_r obtained with the nonlinear regressions of Fig. 8 [calculated with Eqs. (7)] with the true value g_m calculated with Mie theory. Table 1 shows that the anisotropy factor for Mie scatterers can be recovered with the Reynolds–McCormick phase function. Table 1 also demonstrates that the nonlinear regression yields g values with smaller uncertainties if g is higher.

C. Determination of the Scattering Properties of Intralipid and Blood

For determining the scattering coefficient of Intralipid–20%, its concentration was changed by dilu-

Table 1. Comparison of the Anisotropy Factors Calculated from Mie Theory g_m and from Nonlinear Regression g_r the Reynolds–McCormick Phase Function for Polystyrene Spheres and Blood[a]

	Anisotropy Factors	
Scattering Medium	g_m	g_r
Polystyrene spheres		
$d = 300$ nm	0.428	0.41 ± 0.04
$d = 519$ nm	0.742	0.74 ± 0.01
$d = 806$ nm	0.861	0.87 ± 0.01
Blood		
$d = 5.58$ µm	0.9937	0.993 ± 0.001

[a]The uncertainties in g_r originate with the nonlinear-regression procedure.

tion with distilled water until the integrated Doppler spectrum equaled that of the calibration spectrum, as described in Section 3.B. Then a nonlinear regression was applied to this spectrum by the use of the fast Monte Carlo code and the Reynolds–McCormick phase function to derive the anisotropy factor. Figure 9 shows an example of the measurements (solid curve) and of the results after the nonlinear regression (dashed curve).

Five measurements were taken in this way, yielding a scattering coefficient of $\mu_s = 0.58 \pm 0.04\ \text{mm}^{-1}$ for 1 vol. % Intralipid–20% (i.e., 0.2% solids) and an anisotropy factor of $g = 0.72 \pm 0.03$. From this we get $\mu_s' = 0.162 \pm 0.029\ \text{mm}^{-1}$. This value is in accordance with investigations by Wilson *et al.*,[16] who measured a reduced scattering coefficient of $\mu_s' \approx 0.17\ \text{mm}^{-1}$ at 820 nm and by Fantini *et al.*[19] who got $\mu_s' \approx 0.176 \pm 0.010\ \text{mm}^{-1}$ at 850 nm. Both groups used frequency–domain measurement setups.

The same procedure was applied for the measurement of blood diluted with an isotonic NaCl solution. In Fig. 10 one example of a measurement of the Doppler spectrum of blood and a nonlinear regression are shown. Five measurements were conducted, yielding a scattering coefficient of $\mu_s \approx 5.5\ \text{mm}^{-1}$ for blood with a hematocrit of $H = 0.01$ and an anisotropy factor of $g = 0.993 \pm 0.001$. The scattering coefficient could be determined only approximately because frequencies less than 100 Hz cannot be measured with our apparatus. A great fraction of the Doppler-shifted photons are located in this frequency range because blood scatters mainly in the forward direction.

Mie theory yields a scattering coefficient of 4.6 mm^{-1} for blood with a value of $H = 0.01$, assuming that the red blood cells have a spherical shape with a diameter of $d = 5.58\ \mu\text{m}$. Steinke and Shepherd[2] measured the collimated transmission of diluted blood at $\lambda = 632.8$ nm and found that the scattering coefficient was in accordance with Mie theory.

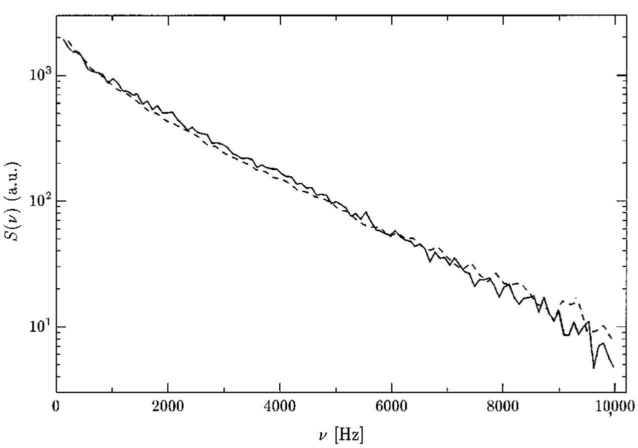

Fig. 9. Plots of the laser Doppler spectra $S(\nu)$ for diluted Intralipid–20% as the moving medium. Shown are experimental results (solid curve) and results from a nonlinear regression (dashed curve) that applied the Reynolds–McCormick phase function in the fast Monte Carlo code.

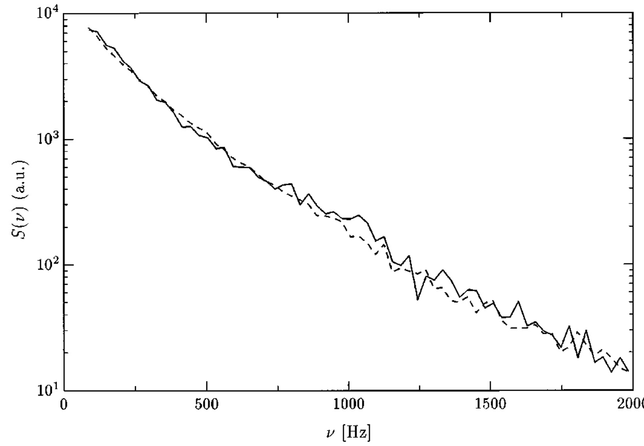

Fig. 10. Plots of the laser Doppler spectra $S(\nu)$ for blood as the moving medium. Shown are experimental results (solid curve) and results from a nonlinear regression (dashed curve) that applied the Reynolds–McCormick phase function in the fast Monte Carlo code.

However, Steinke and Shepherd[2] also found that the anisotropy factor was not well described by Mie theory. At $\lambda = 632.8$ nm they measured a value of $g = 0.9853$, whereas from Mie theory they calculated that $g = 0.9948$. This discrepancy results in a great difference when μ_s' is computed. In contrast to that finding, the anisotropy value measured in the present study is in good accord with that derived from Mie theory: $g = 0.9937$ at $\lambda = 820$ mm. Because the scattering coefficient could be determined only approximately and because knowledge of the scattering coefficient is a prerequisite to determining the anisotropy factor with our method, we varied the concentration of blood in the measurements to assess the sensitivity of g to this uncertainty. Although the concentration of blood was increased and decreased by more than 20%, no significant difference was found in the estimate of g.

5. Discussion

A new approach to the measurement of the anisotropy factor and the scattering coefficient of liquids is presented. The method was validated with measurements on well-characterized suspensions of polystyrene spheres. In addition, the scattering properties of Intralipid–20% were determined and found to be in accord with values from the literature.

Measuring the laser Doppler spectra of liquids that flow through a static scattering medium has the advantage that the anisotropy factor of liquids with forward peak phase functions can be determined accurately: We showed both theoretically and experimentally that the anisotropy factor of blood can be deduced within an error of approximately ± 0.001. It was also found that the measured anisotropy factor of blood is in accordance with Mie theory. In contrast to this, Steinke and Shepherd[2] found that their measured anisotropy factor was smaller than that calculated with Mie theory, but this result was probably due to errors associated with their goniometric technique.

For blood the scattering coefficient could be deduced only approximately because a large fraction of the Doppler spectrum was located at frequencies less than 100 Hz, which could not be measured with our device. This problem could easily be overcome by appropriate modification of the electronics.

In this study, all fluids had an absorption coefficient that was much smaller than the scattering coefficient, which permitted the use of the same probability distribution for the number of scattering events in the moving medium. If there is no *a priori* information about the absorption coefficient, an additional measurement should still permit the determination of the scattering coefficient and the anisotropy factor. For example, collimated transmission through a liquid can easily be measured and provides the extinction coefficient, $\mu_t = \mu_s + \mu_a$. With this knowledge the scattering coefficient can be deduced with the full Monte Carlo program (Section 3.A) and a change in the absorption coefficient (and thus the scattering coefficient, so that the extinction coefficient remains the same) until the integrated Doppler spectrum fits the experimental data. From this simulation, which fits the integrated data, the calculated probability distribution of the number of scattering events can be used to derive the anisotropy factor of the liquid, as described above.

With the techniques presented in this study it is also possible to measure changes in the anisotropy factor rapidly. Assuming that the laser Doppler spectra can be measured within a second, the determination of the anisotropy factor would need approximately the same amount of time if a neural network were trained to fit the experimental data.[20,21]

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