Optical properties of platelets and blood plasma and their influence on the optical behavior of whole blood in the visible to near infrared wavelength range

Martina Meinke
Gerhard Müller
Charité - Universitätsmedizin Berlin
Campus Benjamin Franklin
Institut für Medizinische Physik und Lasermedizin
Fabeckstr. 60-62
14195 Berlin, Germany
E-mail: martina.meinke@charite.de

Jürgen Helfmann
Moritz Friebel
Laser- und Medizin-Technologie GmbH, Berlin
Fabeckstr. 60-62
14195 Berlin, Germany

Abstract. The optical parameters absorption coefficient, scattering coefficient, and the anisotropy factor of platelets (PLTs) suspended in plasma and cell-free blood plasma are determined by measuring the diffuse reflectance, total and diffuse transmission, and subsequently by inverse Monte Carlo simulation. Furthermore, the optical behavior of PLTs and red blood cells suspended in plasma are compared with those suspended in saline solution. Cell-free plasma shows a higher scattering coefficient and anisotropy factor than expected for Rayleigh scattering by plasma proteins. The scattering coefficient of PLTs increases linearly with the PLT concentration. The existence of physiological concentrations of leukocytes has no measurable influence on the absorption and scattering properties of whole blood. In summary, red blood cells predominate over the other blood components by two to three orders of magnitude with regard to absorption and effective scattering. However, substituting saline solution for plasma leads to a significant increase in the effective scattering coefficient and therefore should be taken into consideration. [DOI: 10.1117/1.2435177]

Keywords: optical parameters; absorption coefficient; scattering coefficient; platelets; leukocytes; plasma.

Paper 06060RR received Mar. 10, 2006; revised manuscript received Nov. 14, 2006; accepted for publication Nov. 14, 2006; published online Feb. 2, 2007.

1 Introduction

Investigation of the optical properties of platelets (PLTs) are of interest for hemocytometry and for the control of aggregation and activation.[1,2] For an optical quality control of blood products such as plasma and PLT concentrates,[3,4] knowledge of the optical properties could encourage the development of optical testing methods.

The optical properties of blood are determined by measuring the absorption and scattering of blood cells and plasma components. Data about the optical properties of blood are important not only for many diagnostic and therapeutic applications in laser medicine but also for routine medical diagnostics. They are required for the calculation of the light distribution in blood-perfused tissues, for example, in optical tomography, fluorescence diagnosis, diaphanoscopy, photodynamic therapy, and laser-induced thermotherapy.[5] According to the transport theory, light distribution can be described by the optical parameters absorption coefficient μ_a, scattering coefficient μ_s, and the scattering phase function represented by the anisotropy factor g. The optical parameters of red blood cells for circulating human blood have been determined using integrating sphere measurements and subsequently applied inverse Monte Carlo simulations.[6–8] As far as we know, the optical parameters μ_a, μ_s, g, and μ_s' of plasma and PLTs are not currently available.

Due to their availability and "easy handling," human erythrocyte concentrates are often used instead of whole blood. Normal human whole blood consists of about 55% plasma (90% water, 10% proteins) and 45% cells (99% red blood cells, 1% leukocytes and PLTs). The red blood cells (RBCs) are largely responsible for the optical behavior of blood. They are the most prevalently occurring cells in blood and contain a strongly absorbing metalloprotein, hemoglobin, with a highly complex refractive index,[9] which influences the absorption and scattering properties. The use of RBCs instead of whole blood reduces interfering influences such as protein adsorption on surfaces[10] and aggregation of the RBCs (Ref. 11). This paper presents the optical parameters of blood components such as PLTs and plasma, which were investigated to show their effect on the optical properties of whole blood. The influence of the refractive index of the cell surrounding media was also investigated by comparing the results of the RBCs and PLTs, suspended in saline solution and in plasma. Finally, the optical effect of addition of leukocytes to a PLT plasma suspension was investigated.

An improved integrating sphere technique combined with inverse Monte Carlo simulations[6] was used to determine the μ_a, μ_s, and g of the blood components. The concentration of PLTs in normal whole blood is between 150,000 and 440,000/

Address all correspondence to Martina Meinke Institut für Medizinische Physik und Lasermedizin, Charité - Universitätsmedizin Berlin, Campus, Benjamin Franklin, Fabeckstrasse 60-62 - Berlin, 141195 Germany; Tel.: 49-30-844 54158; Fax: 49-30-844 51289; E-mail: martina.meinke@charite.de

1083-3668/2007/12(1)/014024/9/$25.00 © 2007 SPIE

μL and that of leukocytes is in the range of 4000 to 9000/μL. To avoid aggregation and sedimentation, the cells were investigated under controlled flow conditions. An experimental setup was used that gave transmittance and reflectance measurements in the wavelength range from 350 to 1100 nm under defined flow conditions using a custom-built flow cuvette combined with a miniaturized extracorporal circulation unit.[12]

2 Material and Methods

2.1 *Blood Preparation*

Fresh human plasma concentrates were centrifuged to remove remaining blood cells (leukocytes $<1\times10^6$/unit; PLTs $<5\times10^{10}$/L, and RBCs $<6\times10^9$/L). The first sample (plasma 1) was centrifuged at 62,000 g for 1 h (Allegra 64R, Beckman Coulter) and the second sample (plasma 2) at 1600 g for 30 min (Labofuge A, Heraeus Christ). After centrifugation, no cells could be detected in plasma 1 or were close to the detection limit, as in plasma 2. The cell concentration was measured using a clinical blood analyzer (Micros 60 OT 18, ABX Diagnostics, Montpellier, France). The linear range of the blood analyzer is 0.5 to $80\times10^3/\mu$L for leukocytes, 0.2 to $7.5\times10^6/\mu$L for RBCs, and 10 to $1000\times10^3/\mu$L for PLTs. Highly concentrated suspensions were diluted and PLT concentrations lower than $1\times10^3/\mu$L could not be detected.

A fresh leukocyte-depleted platelet concentrate from a human donor was used. The PLTs were suspended in plasma with a concentration of $1075\times10^3/\mu$L, and were diluted with plasma 1 to different concentrations 5 to $100\times10^3/\mu$L. To investigate the influence of the surrounding media, the PLT sample $1075\times10^3/\mu$L was carefully centrifuged (10 min at 1800 rpm) and the plasma was substituted for 0.9% saline solution. The PLT concentration was reduced by this procedure to $674\times10^3/\mu$L possibly due to residual PLT in the supernatant which was removed from the sample.

The leukocytes were also suspended in plasma. The leukocyte sample included 4500/μL leukocytes and $465\times10^3/\mu$L PLTs. The measurements of the leukocytes sample were compared to a PLT sample of the same concentration without leukocytes. PLTs were investigated within 48 h because of their short lifetime, and all measurements on leukocytes were carried out within 24 h after the cells were received from the blood bank.

The influence of the surrounding media of erythrocytes was investigated after taking RBCs from cell concentrates taken from human healthy donors and washing them three times with 0.9% saline solution. Two samples were prepared with hematocrit 8% (RBC $1.0\times10^6/\mu$L) and 40% (RBC $4.8\times10^6/\mu$L), respectively. Half of the sample volume was centrifuged again (10 min/1600g) and the buffer was substituted for plasma 2 without changing the hematocrit.

2.2 *Experimental Setup*

The double integrating sphere technique combined with inverse Monte Carlo simulation (iMCS) has already been shown to be useful for determination of the optical parameters of blood.

The diffuse reflectance R_d^M, the total transmission T_t^M, and the diffuse transmission T_d^M of thin samples were measured (350 to 1100 nm) using an integrating sphere spectrometer (Lambda 900, PerkinElmer, Rodgau-Jügesheim, Germany), which was described in detail by Meinke et al.[12] A miniaturized extracorporal circulation system was set up to enable the blood to flow under controlled conditions. A special flow-through turbulence-free cuvette was used providing laminar flow up to shear rates of 3000 s^{-1}, enabling the use of a homogeneous rectangular measurement spot of 7×11.5 mm. The sample thickness was adjusted using a spacer with the required thickness. For the measurement of plasma and PLTs, the sample thickness was set to 2.6 mm. Leukocytes were measured using a sample thickness of 0.52 mm and because of the high absorption of hemoglobin, a value of 0.116 mm was selected for the RBCs. The flow was adapted to the thickness to provide a constant wall shear rate of 600 s^{-1} at the cuvette glass surface. The blood temperature was kept constant at 20°C and the pH at 7.4.

2.3 *Evaluation of Optical Properties*

The optical parameters μ_a, μ_s, and g were calculated[6,13] by iMCS. The iMCS uses forward Monte Carlo simulations iteratively to calculate the optical parameters μ_a, μ_s, and g on the basis of a given phase function and the experimentally measured values for reflection and transmission (R_d^M, T_t^M, and T_d^M). The iMCS uses an initial set of μ_a, μ_s, and g to calculate the resulting simulated reflectance and transmission values R_d^S, T_t^S, and T_d^S. These are then compared to the R_d^M, T_t^M, and T_d^M values that have been measured experimentally. By systematic variation of μ_a, μ_s, and g, the deviation of the simulated R_d^S, T_t^S, and T_d^S values from the measured ones are minimized until a set of optical parameters is found where the deviations are within an error threshold of 0.10%.

For realistic Monte Carlo simulations of photon transport in turbid media, the selection of an appropriate effective scattering phase function that is suited to the investigated medium is very important. The Reynolds-McCormick (RM) phase function[6,8] and the Henyey-Greenstein (HG) phase function[14,15] have already been discussed for the scattering of RBCs. As described by Friebel et al.,[6] the best effective phase function can be evaluated using a special iMCS. When using the RM phase function, an optimal value for the alpha factor can be derived that can be used to vary the phase function. In this investigation the RM phase function was tested with $\alpha=0.5$ (corresponds to HG) to $\alpha=3$.

The refractive index of the surrounding medium must be known for calculation of the radiation transport within the Monte Carlo simulation as well as for comparing Mie calculations. Therefore, measurements of the real part of the refractive index of plasma at 400, 500, 600, and 700 nm were carried out using an Abbe Refractometer (Carl Zeiss, Oberkochen, Germany).

3 Results

3.1 *Optical Parameters of Plasma*

The refractive indices of plasma were calculated to be 1.3577 at 400 nm, 1.3506 at 500 nm, 1.3473 at 600 nm, and 1.3438 at 700 nm. The Sellmeier approximation with two parameters was used to fit the refractive index of plasma from 350 to 1100 nm. The calculated refractive indices of plasma were used within the iMCS for the refractive indices of the blood

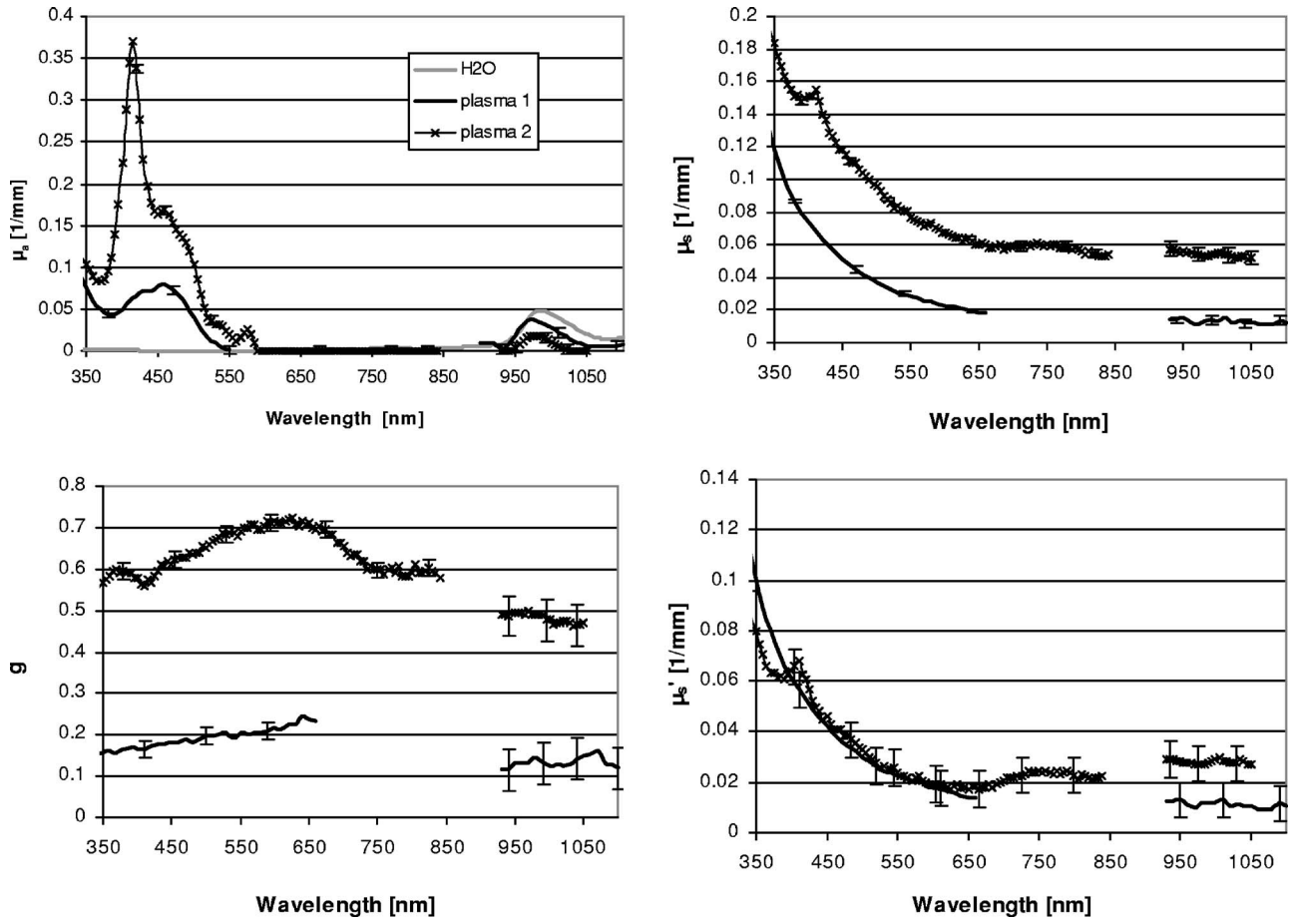

Fig. 1 Absorption coefficient μ_a, scattering coefficient μ_s, anisotropy g, and effective scattering coefficient μ_s' of two different plasma samples.

samples containing plasma as medium. For the samples containing saline solution, the refractive index of water was used for the iMCS as the refractive index of the samples.

The RM phase function, where α is between 1 and 2, was estimated to be the most appropriate effective scattering phase function for human plasma in the range of 350 to 1100 nm. The HG phase function led to 59% of erroneous simulations with an error larger than 0.05%. Using the RM phase function, with α=1.5, there is an error occurrence of only 6%. Figure 1 shows the optical parameters, calculated using the iMCS, with the evaluated effective phase function using α =1.5 after measurement of R_d, T_t, and T_d of two plasma samples. Plasma 1 was colorless with no detectable cell concentration. Plasma 2 had a PLT concentration of about 1000/μL and a slight orange color indicative of a different absorption spectrum. In some cases, the absorption and scattering properties of the plasma samples were too low for the wavelength ranges and this resulted in unsuccessful simulations. These data have been removed from the figures. The error bars are standard deviations (SDs) for selected wavelengths resulting from preparation, measurement, and simulation errors. For μ_a, the SD is 0.005 mm^{-1}. The SD for μ_s is between 0.001 and 0.003 mm^{-1}, the SD for g ranges from 0.02 to 0.05, and for μ_s' the SD is estimated to be 0.007 mm^{-1}.

The absorption coefficient μ_a of plasma depending on the wavelength in Fig. 1, upper left panel, is shown in direct comparison to the absorption coefficient of water. Plasma 1 shows an absorption peak at about 450 nm, with a maximum at 460 nm, reaching a value of 0.08 mm^{-1}. Plasma 2 shows an absorption peak at 415 nm of 0.37 mm^{-1} and additionally at 580 nm of 0.02 mm^{-1}. Above 750 nm, absorption of plasma generally follows that of water. The absorption peak of water at 990 nm is smaller and shifted to 975 nm.

The scattering behavior of plasma is illustrated in Fig. 1, upper right and the lower panels. For plasma 1 the scattering coefficient μ_s decreases continuously from 0.12 mm^{-1} at 350 nm to 0.013 mm^{-1} at 1100 nm. The anisotropy factor g in the spectral range of 350 and 1100 nm is between 0.12 and 0.25. The curve of the effective scattering coefficient μ_s' $=\mu_s(1\text{-}g)$ behaves similarly to μ_s and decreases from 0.1 mm^{-1} at 350 nm to below 0.02 mm^{-1} above 600 nm.

The plasma sample 2 shows different optical behavior characteristics, where μ_s is significantly higher, about 0.05 mm^{-1}, over the whole spectrum. The g factor is also higher with values between 0.5 and 0.7, resulting in an effective scattering coefficient μ_s' comparable to that obtained for plasma 1.

3.2 *Optical Properties of Platelets in Plasma*

In whole blood, as well as in PLT concentrates, provided by the Department of Transfusion Medicine, PLTs are always

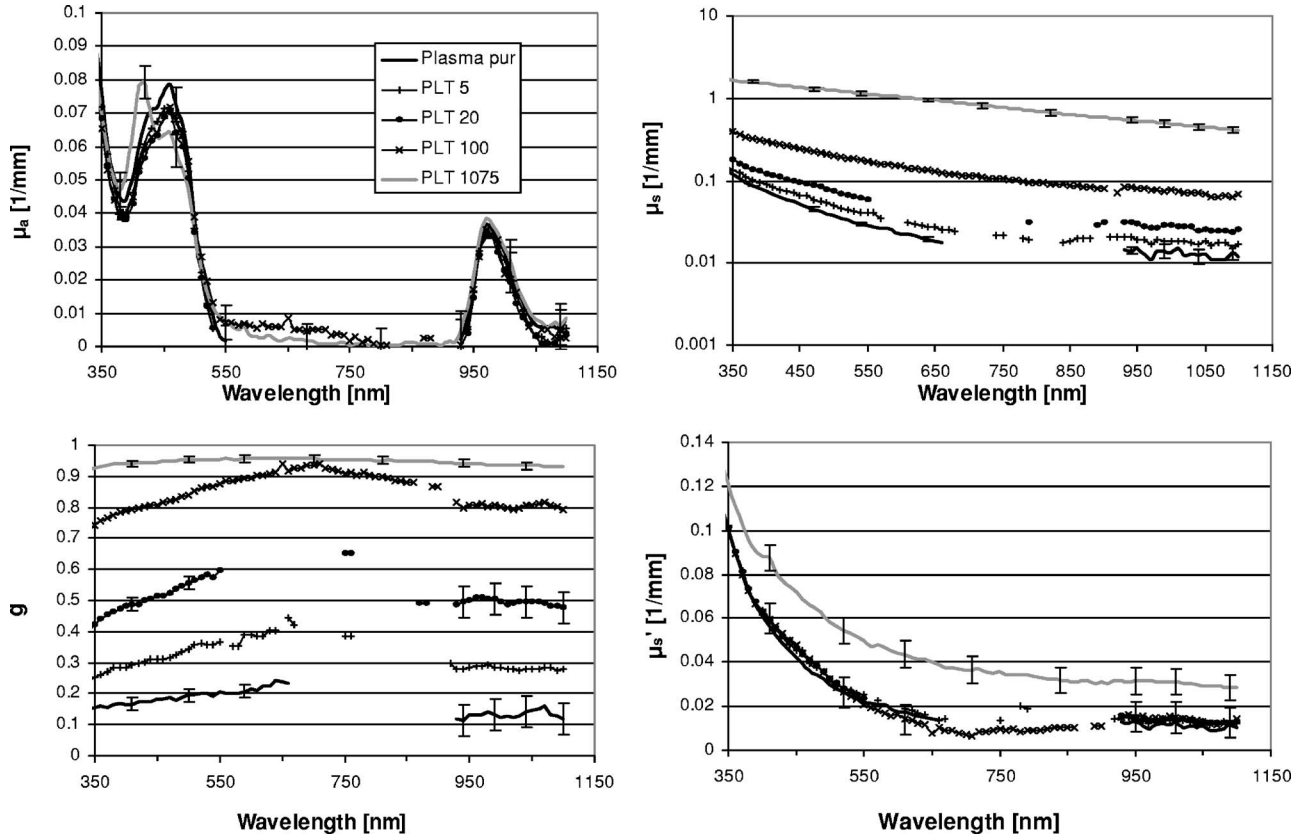

Fig. 2 Absorption coefficient μ_a, scattering coefficient μ_s, anisotropy g, and effective scattering coefficient μ_s' of different amounts of platelets (concentration in $10^3/\mu$L) in plasma compared to the corresponding plasma.

suspended in plasma. The RM phase function with α between 1 and 2 was evaluated as having the most effective phase function for the PLTs plasma suspension. The RM phase function was used for all PLT samples with $\alpha=1.5$ because only 3% simulation errors occurred in contrast to the HG phase function with a total of 30% simulation errors.

Figure 2 shows the optical parameters of various concentrations of PLTs in plasma calculated using the iMCS compared to pure plasma. The mean standard deviations of μ_a, μ_s, g, and μ_s' correspond to those of the plasma measurements. The PLTs in plasma have an absorption behavior very similar to that of pure plasma, indicating that additional cells do not change the absorption significantly. Only if the PLTs are highly concentrated is the maximum absorption shifted to 420 nm (0.079 mm^{-1}). The scattering coefficient decreases with wavelength and increases linearly with PLT concentration. The g factor increases with wavelength up to 600 nm. Above 600 nm, g shows a slight decrease. Furthermore, g increases for all wavelengths with increasing PLT concentration, for example, at 500 nm from 0.2 for pure plasma over 0.34 for 5×10^3 PLT/μL to 0.953 for 1075×10^3 PLT/μL. The scattering coefficient decreases with wavelength. Starting from the μ_s' of plasma, μ_s' increases with increasing PLT concentration. Due to the error of measurement, the values for μ_s' at low PLT concentrations do not show a significant difference to those of plasma. The μ_s' values of the highest PLT concentration are twice as high compared to pure plasma and reach 0.121 mm^{-1} at 350 nm and 0.028 mm^{-1} at 1100 nm.

3.3 *Optical Properties of Leukocytes in Plasma*

The leukocytes, provided by the Department of Transfusion Medicine, were suspended in plasma containing PLTs of a concentration of 465×10^3 PLT/μL.

With regard to the error of measurement, no significant differences in absorption or scattering properties appear to have been caused by adding a physiological amount of leukocytes (4.5×10^3 /μL) to the PLT suspension in plasma. Therefore no figures are presented for these data.

3.4 *Influence of the Surrounding Medium*

Figure 3 shows the comparison of platelet suspended in 0.9% saline solution (674×10^3 PLT/μL) to PLT in plasma (1075×10^3 PLT/μL). The mean standard deviations of μ_a, μ_s, g, and μ_s' correspond to those of the plasma measurements. The absorption of the saline solution suspension is significantly lower at the absorption peak at 450 nm with values of 0.01 mm^{-1}. Although the PLT concentration of the saline solution is lower by a factor of 1.59, the scattering coefficient is higher compared to the plasma sample. The μ_s of PLT in saline solution is 2.18 mm^{-1} at 350 nm, which is a factor of 1.3 times higher than the μ_s of the more concentrated plasma suspension. PLTs in saline solution show a higher g factor over the whole spectrum than PLTs in plasma. The g factor of PLTs in saline solution varies slightly over the wavelength range: 0.963 at 350 nm, 0.967 at 700 nm, and 0.953 at 1100 nm, whereas the g factor for PLTs in plasma is

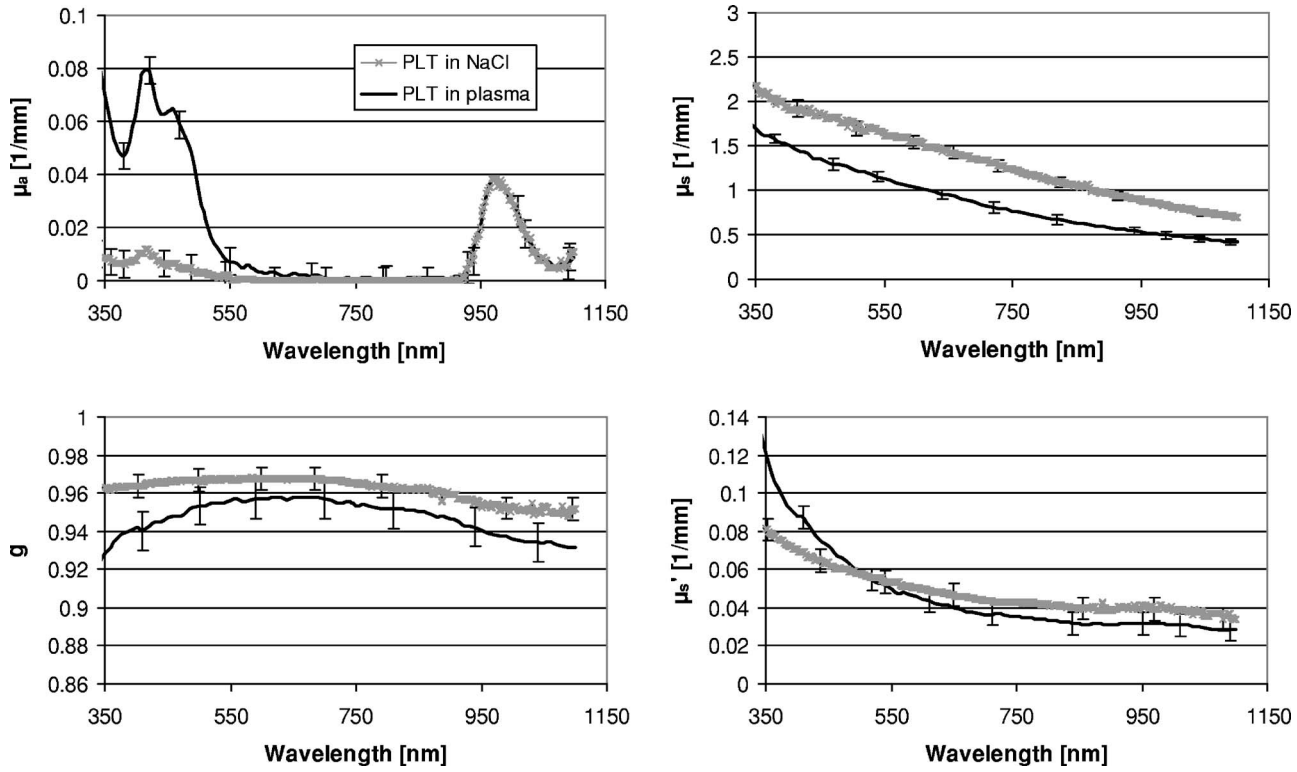

Fig. 3 Optical parameters μ_a, μ_s, g, and μ_s' of $1075\times10^3/\mu$L platelets in plasma and $674\times10^3/\mu$L platelets in 0.9% sodium chlorine solution.

lower by about 0.012 but decreases more drastically for short wavelengths going down to 0.926 at 350 nm. As a consequence, the μ_s' of PLTs in plasma exceeds the one in saline solution from 350 to 500 nm. Above 550 nm, μ_s' for PLTs in saline solution is approximately 0.008 mm^{-1} higher than that in plasma.

In Fig. 4 the optical parameters are shown for RBCs suspended in plasma and saline solution. The cell concentrations amount to $1.0\times10^6/\mu$L for both samples. This corresponds to a hematocrit (Hct) of 8%. For the μ_a of RBCs in saline solution, the mean relative SD is 2.2%. The mean relative SDs for μ_s and g are 1.6 and 0.056%, respectively, and for μ_s' they are in the range of 1.8 to 7%. The SD for the optical properties of the plasma samples is slightly higher. The Soret absorption at 415 nm and also the two absorption maxima at 542 and 577 nm of the RBCs in saline solution exceed that of RBCs in plasma. The μ_s of RBCs in saline solution is about 4 mm^{-1} on average higher than of RBCs in plasma over the whole wavelength range. Up to 490 nm, the g factor of the cells in saline solution is higher than that in plasma, whereas above this wavelength, g is, on average, 0.004 lower. The effective scattering coefficient of RBCs in saline solution is between 0.2 to 0.4 mm^{-1} higher compared to RBCs in plasma. Investigations of RBCs at a concentration of $4.8\times10^6/\mu$L (Hct 40%)—not shown here—show similar results but the differences between RBCs in the two media decrease relatively.

4 Discussion

4.1 *Optical Properties of Plasma*

The absorption of plasma in the near IR is dominated by the water absorption. In the UV to visible range, plasma shows a significant absorption due to the chromophores found in proteins and other molecules. The plasma absorption exhibits a wide individual variability brought about by various protein concentrations, nutritive compounds, or pharmaceuticals, for example, contraceptives. Even the visual assessment of plasma samples from different donors shows a variety of colors ranging from yellow to green to orange to brown and their transitions. The absorption peak of plasma 1 at about 450 nm can be associated with the bilirubin absorption. Often the most prominent absorption peak is at 415 nm, which is due to the remaining RBCs or free hemoglobin from the plasma production. Furthermore, scattering particles, such as lipids, can result in turbid plasma samples that are usually excluded in routine plasma quality control. This means that the results shown here are exemplary.

The scattering properties of pure plasma are described by Rayleigh scattering of protein molecules, resulting in a scattering cross section that decreases with increasing wavelength ($\sim\lambda^{-4}$) and isotropic scattering corresponding to a g factor of zero. For plasma 1, the g factor is about 0.2 in the investigated wavelength region, indicating a slightly forward scattering, and μ_s is much higher than expected by Rayleigh scattering, calculated for a 10% albumin solution. On the basis of calculations according to Mie theory, it is estimated that particles with a size of approx. 100 nm and a total concentration of 10% must be present to explain the deviation from Rayleigh scattering. In plasma 1, these particles might be protein aggregates and lipids or cell components which could not be removed by the given centrifugation procedure.

In plasma 2, residual RBCs and PLTs would be a plausible explanation for the relatively high absorption at 415 nm and the higher g value compared to plasma 1. This could lead to

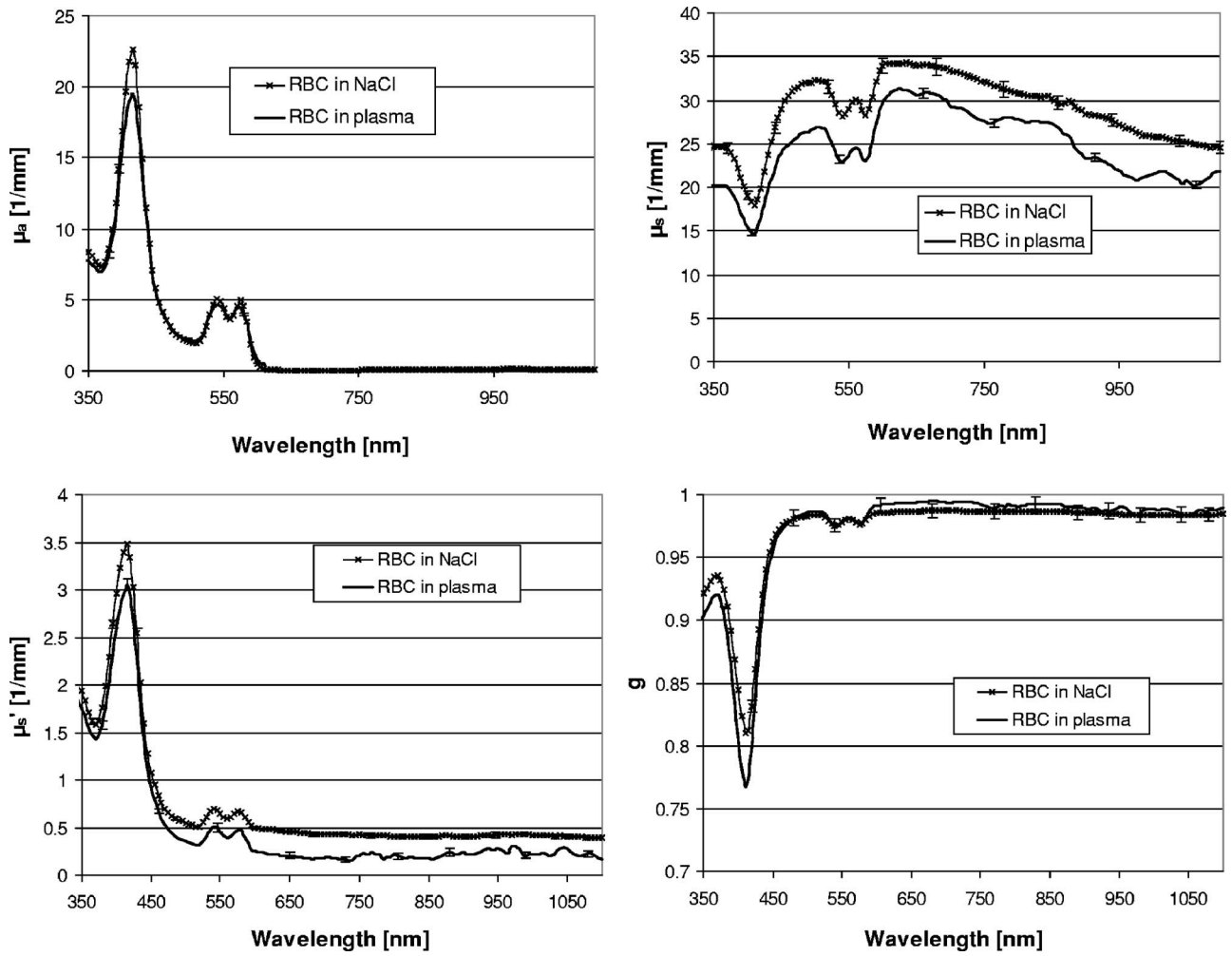

Fig. 4 Optical parameters μ_a, μ_s, g, and μ_s' of $1\times10^6/\mu$L red blood cells in plasma and in 0.9% sodium chlorine solution, corresponding to a Hct of 8%.

the assumption that the plasma samples are composed of different constituents such as molecules, aggregates of molecules, or cells of different sizes. The absorption or scattering of samples is given by the sum of the absorption coefficients μ_a^C or by the sum of scattering coefficients μ_s^C of each compound,

$$\mu_a^C = \mu_a^1 + \mu_a^2, \tag{1}$$

$$\mu_s^C = \mu_s^1 + \mu_s^2. \tag{2}$$

According to the continuum character of the transport theory, a composed phase function and, as a consequence, a composite anisotropy factor g^C can be derived for a scattering medium consisting of two different scatterers. In this case, the single anisotropy factors are averaged and weighted by the scattering coefficients of the respective compound:

$$g^c = \frac{\mu_s^1 g_1 + \mu_s^2 g_2}{\mu_s^1 + \mu_s^2}. \tag{3}$$

This could explain the relatively large difference between the g factors of plasmas 1 and 2. Whereas g of plasma 1 is close to the anisotropy of the pure plasma, the g factor of plasma 2 is a composite g factor, increased by a significant amount of remaining cells of which the g factors are close to 1.

4.2 *Optical Properties of Platelets*

As shown in Fig. 2, the addition of a physiological amount of PLTs does not change the absorption of plasma. The plasma mainly determines the absorption. This could be demonstrated by substitution of plasma by saline solution. The absorption between 350 and 500 nm is reduced by up to a factor of 10 even when there is residual plasma.

The linear increase of μ_s of PLTs in plasma with increasing PLT concentration suggests that measurement of the amount of PLTs would be possible by reflectance measurements. The composite g factor of PLTs in plasma is lowered when the PLT concentration decreases because the influence of the low plasma g factor increases. Development of devices for the determination of low PLT concentrations would be required to account for the changes in g because the effective scattering coefficient does not change significantly in concentrations up to 100×10^3 PLTs/μL.

4.3 *Influence of the Medium*

The scattering properties of particles suspended in a medium depend on the difference in their refractive indices and, therefore, on the refractive index of the medium. The refractive index of PLTs is higher than that of plasma. Mattley et al.[16] determined the refractive index of PLTs to be 1.39 at 600 nm and that of plasma to be $n_{plas}=1.35$. The latter is in good agreement with the Abbe refractive index measurement of plasma determined in this study to be $n_{plas}=1.347$ at 600 nm. This is significantly higher than that of water[17,18] ($n_{H_2O}=1.333$), which is not supposed to differ from the index of a 0.9% saline solution. The refractive index of RBCs is higher than that of PLTs: $n_{Hb}=1.417$ at 600 nm for an intracellular hemoglobin concentration[19] of 32 g/dL.

Therefore, the change in the scattering properties of PLTs or RBCs, when plasma is replaced by saline solution, is a result of an increase in the difference between the refractive indices of the cells and the medium. A calculation with Mie theory for spheres of the same volume and refractive index as PLTs or RBCs shows an increase of the scattering cross section and a decrease in the anisotropy factor when replacing plasma by saline solution. If the medium has absorption and scattering properties itself, as is the case for plasma, the absorption coefficient μ_a^C, the scattering coefficient μ_s^C and the g factor are made up as shown in Eqs. (1)–(3).

As a consequence, changes in refractive indices, as well as in the composition of the optical parameters, are two different effects and both should be considered when comparing cells in saline solution with cells in plasma. One effect can dominate the other, dependent on the quantities of the optical parameters.

The scattering coefficients of PLTs and RBCs in saline solution are higher than those of the cells in plasma, indicating a predominating effect of the refractive index change.

The g factor of PLTs in saline solution is higher than that in plasma, indicating that the very low plasma g factor has led to a significant decrease in the composite g factor of PLTs in plasma. This is because the scattering coefficients of plasma are nonnegligible compared to those of PLTs. This effect is more dominant for short wavelengths where the plasma μ_s has more influence compared to the PLT μ_s, due to the predominating exponential increase (λ^{-4}) with decreasing wavelength. As a result, μ_s' of PLTs in plasma for short wavelengths exceeds the one in saline solution, but over 500 nm the effective scattering of PLTs in saline solution is higher.

The two effects for the g factor of RBCs, as already discussed, are in direct competition with one another. Below 600 nm, the g factor of RBCs in saline solution exceeds that of RBCs in plasma, as found for the whole wavelength range for PLTs. This effect is due to the low g factor and relatively high μ_s for plasma, and the low g factor and relatively low μ_s for RBCs in this wavelength region resulting in a lower composite g value for RBCs in plasma. For wavelengths over 600 nm, the smaller difference in refractive index of RBCs and plasma is the dominant effect, resulting in a higher composite g factor of RBCs in plasma compared to RBCs in saline solution.

These changes in g and μ_s result in an increase of μ_s' of RBCs in saline solution compared to that of RBCs in plasma over the whole wavelength range. This significant change in the scattering behavior must be considered when using RBC concentrates instead of whole blood for optical investigations.

A general discussion of the optical properties of RBCs can be found in Friebel et al.[6]

4.4 *Influence of Blood Components on the Optical Behavior of Human Whole Blood*

To compare the different blood components, examples of the optical parameters of RBCs, PLTs, and plasma are shown in Fig. 5. The RBCs and the PLTs are at physiological concentration (Hct 40%, 100×10^3 PLTs/μL) and plasma 1 contains almost no residual cells. Data relating to μ_a, μ_s, and μ_s' are shown on a logarithmic scale. The absorption of the RBCs exceeds the others by far. The absorption and the scattering coefficient of the RBCs are two to three orders of magnitude larger than those of PLTs or plasma. This is due to the high refractive index of the RBC.

The g values for RBCs are, on average, higher except for the high absorption of hemoglobin ranging from 300 to 450 nm. All blood cells show strong forward-scattering behavior. The resulting effective scattering coefficients represent two orders of magnitude difference between RBCs and the other blood components. Investigations of RBCs without PLTs and leukocytes, instead of whole blood, would give results with an error of less than 1% for the scattering and absorption properties. Plasma itself shows very small absorption and scattering coefficients compared to RBCs, but its influence as a medium is an important aspect. The differences in refractive index between saline solution and plasma change the scattering properties of the RBCs by a factor of up to two.

In addition to the direct optical effects, the replacement of plasma by saline solution also leads to physiological effects that can significantly influence the optical behavior of blood. Plasma proteins cause the aggregation of RBCs at low shear rates. Plasma has a higher viscosity than saline solutions, leading to different flow conditions which then lead to a change in flow-dependent phenomena such as cell deformation or cell alignment or contribution effects such as axial migration. For example, in the case of high hematocrit levels, the scattering anisotropy of RBCs is greatly influenced by the flow conditions[20–22] particularly the shear rate-dependent aggregation and disaggregation phenomena.[23–25]

Holding the shear rate above the level where aggregation can occur could minimize these physiological influences. Furthermore, the flow velocity of plasma suspensions was diminished to compensate the increase in shear forces induced by the increased viscosity.

Due to the wide variation in the optical properties of plasma, investigations of RBCs should be carried out in saline solutions, which result in a higher level of measurement reproducibility.

5 Conclusion

Blood plasma shows weak absorption and scattering but a higher scattering coefficient and g factor than expected by the Rayleigh scattering of proteins of the size of albumin. The existence of physiological concentrations of leukocytes has no measurable influence on the optical properties of plasma and, therefore, on the optical parameters of whole blood. The addition of PLTs in plasma does not change the absorption but

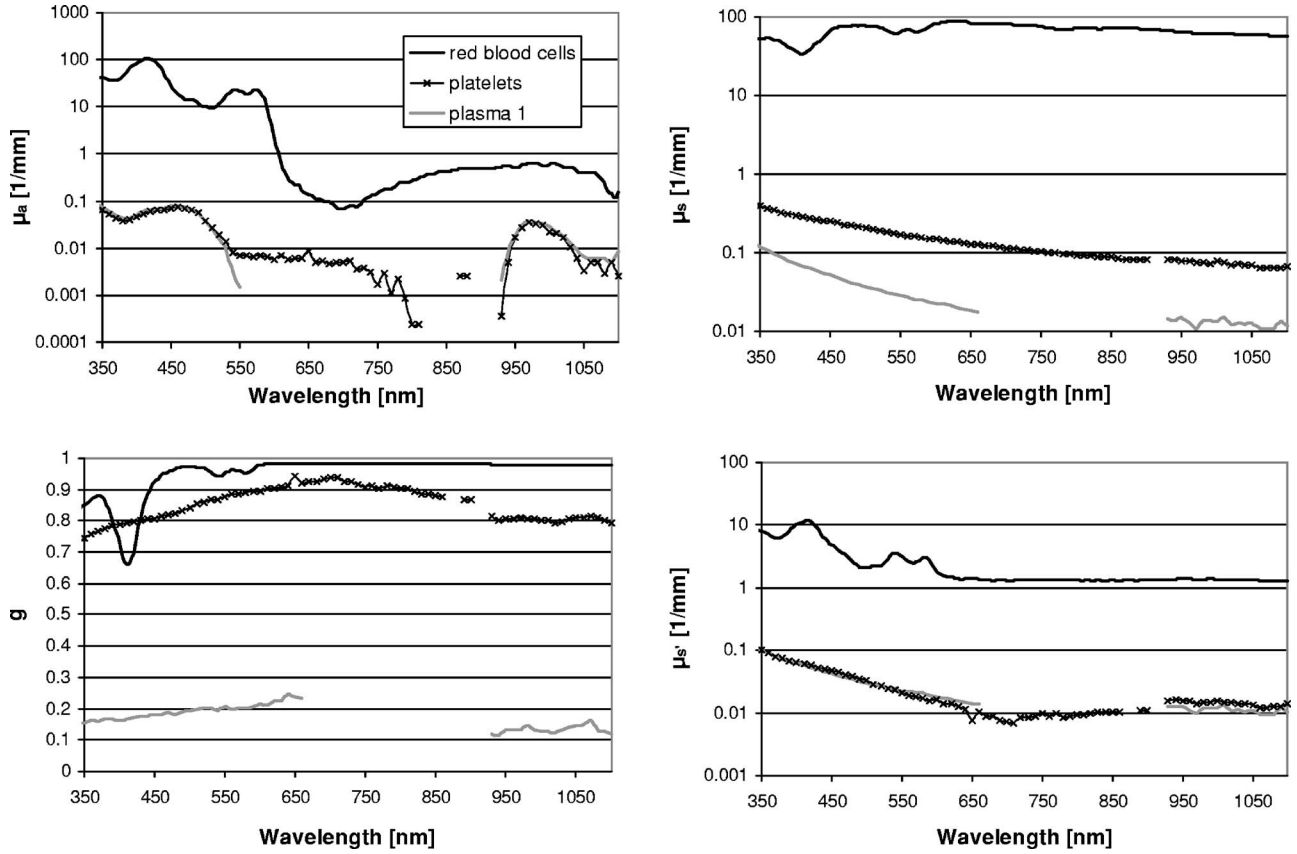

Fig. 5 Comparison of the optical parameters μ_a, μ_s, and g of the various cell compounds of blood: 4.8×10^6 RBCs/μL in plasma corresponding to a Hct of 40%, 1075×10^3 platelets/μL in plasma and cell-reduced pure plasma.

enhances the scattering properties. The scattering coefficient of PLTs in plasma correlates linearly with the PLT concentration.

In summary, the optical properties of RBCs are two to three orders of magnitude larger than those of the other blood components. The influence of plasma as a medium is particularly important. The slightly higher refractive index of plasma compared to that of saline solutions can change the effective scattering of RBCs by up to 100% when substituting plasma by saline solution. This must be considered when making statements about the absolute optical parameters of whole blood from measurements conducted on RBC concentrates as is often used for practical reasons.

Acknowledgments

This work was supported by the Federal Ministry of Education and Research (Grant No. 13N7522). The authors also wish to thank Sorin Group Deutschland GmbH, Munich, for placing the equipment at our disposal. The Department of Transfusion Medicine, Charité-Universitätsmedizin Berlin, Germany, kindly provided the blood bags.

References

1. L. P. Jaremo, C. Sollen, B. Edlund, and O. Berseus, "Correlation of aggregometry responses to changes in light transmission through platelets packs," *Thromb. Res.* **62**(3), 199–207 (1991).
2. L. G. S. Abela, R. Huang, H. Ma, A. R Prieto, M. Lei, A. H. Schmaier, K. A. Schwartz, and J. M. Davis, "Laser-light scattering, a new method for continuous monitoring of platelet activation in circulating fluid," *J. Lab. Clin. Med.* **141**(1), 50–57 (2003).
3. L. T. L. Berg, G. Leparc, D. E. Huffman, A. L Gennaccaro, A. Gracia-Lopez, G. Klungness, C. Soephans, and L. H. Gracia-Rubio, "Detection of microbial contamination in platelets," *Proc. SPIE* **5702**, 76–83 (2005).
4. L. T. Giout, W. Blondel, J. Didelon, V. Latger, D. Dumas, F. Schooneman, and J. F. Stoltz, "Development and evaluation of an automatic method for the study of platelet osmotic response," *Technol. Health Care* **7**(5), 371–380 (1999).
5. L. J. P. Ritz, C. Isbert, A. Roggan, C. T. Germer, D. Albrecht, and H. J. Buhr, "Laser-induced thermotherapy of liver metastasis-evaluation of optical and thermal penetration depth," *J. Cancer Res. Clin. Oncol.* **124**(Suppl.), R83 (1998).
6. L. M. Friebel, A. Roggan, G. Müller, and M. Meinke, "Determination of optical properties of human blood in the spectral range 250–1100 nm using Monte Carlo simulations with hematocrit-dependent effective scattering phase functions," *J. Biomed. Opt.* **11**(3), 031021 (2006).
7. L. A. Roggan, M. Friebel, K. Dörschel, A. Hahn, and G. Müller, "Optical properties of circulating human blood in the wavelength range 400–2500 nm," *J. Biomed. Opt.*, **4**(1), 36–46 (1999).
8. L. A. N.Yaroslavsky, I. V. Yaroslavsky, T. Goldbach, and H. J. Schwarzmaier, "The optical properties of blood in the near infrared spectral range," *Proc. SPIE* **2678**, 314–324 (1996).
9. L. M. Friebel and M. Meinke, "Determination of the complex refractive index of highly concentrated hemoglobin solution using transmittance and reflectance measurements," *J. Biomed. Opt.* **10**(6), 064019 (2005).
10. G. Müller, "Spectroscopy with the evanescent wave in the visible region of the spectrum," in Proc. ACS Symposium Series 102, *Multichannel Image Detectors*, Y. Talmi, Ed., Ch. 12, pp. 239–262 (1979).
11. L. J. Lademann, H. Richter, W. Sterry, and A. V. Priezzhev, "Diag-

nostic potential of erythrocytes aggregation and sedimentation measurements in whole blood," *Proc. SPIE* **4263**, 106–111 (2003).
12. L. M. Meinke, I. Gersonde, M. Friebel, J. Helfmann, and G. Müller, "Chemometric determination of blood parameters using VIS-NIR-spectra," *Appl. Spectrosc.* **59**(6), 826–835 (2005).
13. A. Roggan, O. Minet, C. Schröder, and G. Müller, "Measurements of optical properties of tissue using integrating sphere technique" in *Medical Optical Tomography: Functional Imaging and Monitoring*, G. Müller et al., Eds., Vol. **IS11**, pp. 149–165, SPIE Press, Bellingham, WA (1993).
14. L. A. N. Yaroslavesky, I. V. Yaroslavsky, T. Goldbach, and H. J. Schwarzmaier, "Influence of the scattering phase function approximation on the optical properties of blood determined from the integrating sphere measurements," *J. Biomed. Opt.* **4**(1), 47–53 (1999).
15. L. M. Hammer, A. N. Yaroslavsky, and D. Schweitzer, "A scattering-phase function for blood with physiological haematocrit," *Phys. Med. Biol.* **46**, N65–N69 (2001).
16. L. Y. Mattley, G. Leparc, R. Potter, and L. Garcia-Rubio, "Light scattering and absorption model for the qualitative interpretation of human blood platelets spectral data," *Photochem. Photobiol.* **71**(5), 610–619 (2000).
17. L. D'Ans and E. Lax, *Taschenbuch für Chemiker und Physiker*, Makroskopische physikalisch-chemische Eigenschaften, pp. 627–628, Springer Verlag, Berlin (1967).
18. L. B. Herman, A. J. LaRocca, and R. E. Turner, "Atmospheric scattering," Chap. 4, in *The Infrared Handbook*, W. W. Wolfe and G. J. Zissis, Eds., pp. 4-43–4-45, ERIM, Ann Arbor, MI (1978).
19. L. M. Friebel and M. Meinke, "Model function to calculate the refractive index of native hemoglobin in the wavelength range of 250–1100 nm dependent on concentration," *Appl. Opt.* **45**(12), 2838–2842 (2006).
20. A. H. Gandjbakhche, P. Mills, and P. Snabre, "Light-scattering technique for the study of orientation and deformation of red blood cells in a concentrated suspension," *Appl. Opt.* **33**(6), 1070–1078 (1994).
21. A. Priezzhev, S. G. Khatsevich, and V. Lopatin, "Asymmetry of light backscattering from Couette flow of RBC suspensions:application for biomonitoring of blood samples," *Proc. SPIE* **3567**, 213–222 (1999).
22. V. Lopatin, A. Priezzhev, and V. Feodoseev, "Numerical simulation of light scattering in turbid biological media," *Crit. Rev. Biomed. Eng.* **29**(3), 400–419 (2001).
23. L. G. Lindberg and P. A. Oberg, "Optical properties of blood in motion," *Opt. Eng.* **32**(2), 253–257 (1993).
24. A. M. K. Enejder, J. Swartling, P. Aruna, and S. Andersson-Engels, "Influence of cell shape and aggregate formation on the optical properties of flowing whole blood," *Appl. Opt.* **42**(7), 1384–1394 (2003).
25. V. S. Lee and L. Tarassenko, "Absorption and multiple scattering by suspensions of aligned red blood cells" *J. Opt. Soc. Am. A* **8**(7), 1135–1141 (1991).