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NOTE

A scattering phase function for blood with physiological haematocrit

Martin Hammer[1], **Anna N Yaroslavsky**[2] **and Dietrich Schweitzer**[1]

[1] University of Jena, Department of Ophthalmology, Bachstrasse 18, 07740 Jena, Germany
[2] Wellman Laboratories of Photomedicine, Department of Dermatology, MGH BHX 630, 55 Fruit St, Boston, MA 02114, USA

E-mail: martin.hammer@med.uni-jena.de

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Abstract

Though the optics of red blood cells as well as whole blood has been studied extensively, an effective scattering phase function for whole blood is still needed. The interference of waves scattered by neighbouring cells cannot be neglected in highly concentrated suspensions such as whole blood. As a result, the phase function valid for single erythrocytes may fail to describe a single scattering process in whole blood with physiological haematocrit (Hct $\approx$ 0.4). In this study we compared the results obtained in goniophotometric measurements of blood samples with the results of angle-resolved Monte Carlo simulations. The results show that a Henyey–Greenstein phase function with an anisotropy factor of 0.972 is an adequate approximation for the effective scattering phase function of whole blood with high haematocrit at a wavelength of 514 nm.

1. Introduction

The application of lasers in different areas of medicine requires exact laser light dosimetry. Therefore knowledge of the optical properties of biological tissues, such as the absorption coefficient, the scattering coefficient and the scattering phase function, is of paramount importance for the development of laser medicine.

Since blood strongly absorbs and scatters visible and near-infrared light, its optical properties have been widely investigated. At a microscopic level, the absorption cross section σ_a and the scattering cross section σ_s were determined by Reynolds *et al* (1976), Steinke and Shepherd (1988) and by Hammer *et al* (1998). The macroscopic absorption coefficient μ_a and scattering coefficient μ_s, which are inversely proportional to the respective mean free pathlengths, can be obtained from

$$\mu_a = \frac{H}{V}\sigma_a \qquad \text{and} \qquad \mu_s = \frac{H(1-H)}{V}\sigma_s \tag{1}$$

where V is the volume of a red blood cell and H is the volume fraction of the cells in the sample (haematocrit). These equations, given by Twersky (1970), were verified experimentally

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by Steinke and Shepherd (1986). Phase functions of the optically thin whole blood layers were measured by Flock *et al* (1987), Yaroslavsky *et al* (1996) and Hammer *et al* (1998). However, the scattering phase function of the single red blood cell cannot be adopted for whole blood with a physiological haematocrit ($0.37 < \mathrm{Hct} < 0.54$) without additional verification. As was argued by Reynolds *et al* (1976), Roggan *et al* (1998) and Hammer *et al* (1998), the interference of waves scattered by neighbouring erythrocytes has to be taken into account in densely packed cell suspensions. A theoretical description was published by Uzunoglu *et al* (1995) assuming the local distribution of the dielectric constant of two erythrocytes in the integral representation of scattering (Ishimaru 1978). However, the solution of this integral equation on the basis of the Fredholm's theory was possible only for special geometrical cell arrangements.

The integrating sphere technique, combined with one of the inverse algorithms based on the radiative transport theory, is a powerful tool for characterization of the optical properties of biological tissues *in vitro*. This method has been used to determine optical properties of whole blood with physiological haematocrit by Yaroslavsky *et al* (1996, 1997), Nilsson *et al* (1997) and Roggan *et al* (1998). As was shown by van de Hulst (1980), an accurate description of the scattering phase function is an important issue for media such as blood with a high anisotropy factor. Yaroslavsky *et al* (1999) confirmed that the approximation of the blood scattering phase function is critical for correct determination of its optical properties.

Therefore the goal of the research presented here was to find an effective phase function for densely packed erythrocytes using an empirical approach.

2. Methods

Human whole blood was obtained from healthy donors. The erythrocytes were separated from the blood plasma and the white cell fraction by centrifugation at 3200 rpm for 10 min and were washed three times in an isotonic phosphate buffer ($\mathrm{pH} = 7.4$, $n = 1.33$). The red blood cells were diluted in isotonic phosphate buffer to a suspension with a haematocrit of 0.4. The haematocrit, the haemoglobin concentration and the number and volume of erythrocytes in each sample were determined using a Cell-Dyn 1700 (Abbott, Germany). All scattering experiments were performed with fully oxygenated erythrocytes. To ensure this, the specimens were bubbled with pure oxygen immediately before the measurement.

The goniophotometric measurements were conducted in the following way. The 514 nm light of a continuous-wave argon ion laser ILA190 (Carl Zeiss Jena, Germany) was used as the source. The spot diameter at the sample was 2 mm and the beam divergence was 0.5 mrad. The laser beam was directed onto a quartz cuvette (Helma Optik, Germany) containing the blood sample. The thickness of the cuvette was 100 μm. An attempt to examine flowing blood was not successful since the haematocrit of the blood sample was not sufficiently stable within the laser spot under flow conditions.

The scattered light and the reference beam were measured by a laser power meter Rm 6600 (Laser Probe, NY) equipped with two probes, RkP-575 (1 mW–10 W) and RkP-576 (1 nW–1 mW). The latter was positioned behind a pinhole with a diameter of 1.3 mm at a distance of 55 cm from the cuvette containing the blood sample resulting in an aperture solid angle of 4.4×10^{-6} sr. The detector was rotated around the cuvette with a positioning precision of 0.1°. Measurements of the intensity distribution of the scattered light were taken in the range from 2.5 to 175°. Measured values were corrected for the refractive index mismatch on the water/air interface. The complete angular scan took less than 10 min. No sedimentation of the cells in the cuvette was observed during this time. In order to avoid the influence of ambient light, the incident laser beam was chopped and a lock-in technique was used. Ten measurements

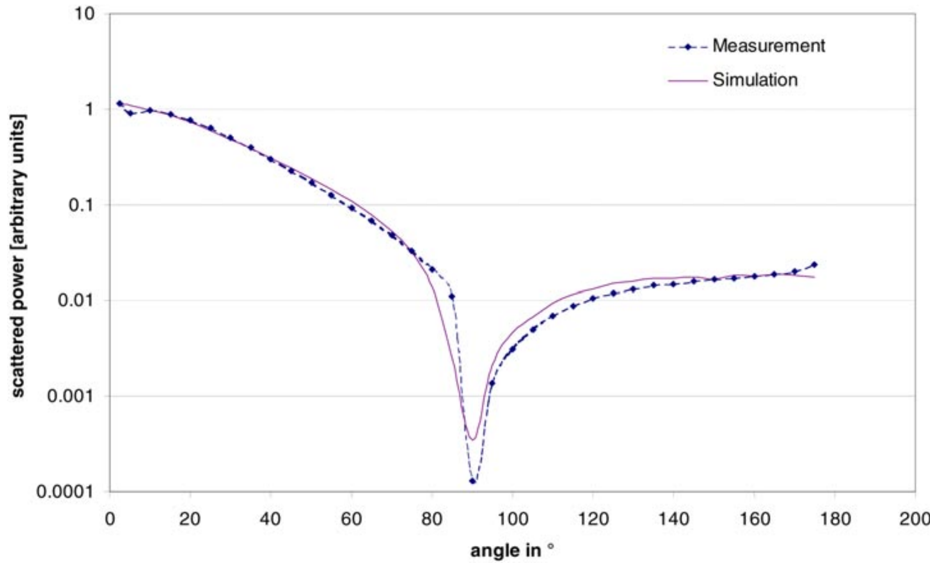

Figure 1. Measured and simulated angular distribution of light power scattered by a layer of whole blood 0.1 mm thick.

were recorded and averaged at every angle. The relative standard deviation was always less than 5%.

The measured angle-resolved intensity of the scattered light was compared with results from the angle-resolved Monte Carlo simulation. The principles of Monte Carlo simulation, as a technique widely used for solving radiative transfer problems, are described elsewhere (Keijzer *et al* 1989, Yaroslavsky and Tuchin 1992, Jacques and Wang 1995). To achieve angular resolution the basic Monte Carlo algorithm was modified as described by Yaroslavsky *et al* (1999). The geometry of the simulation was set up to fit the geometry of the experiment.

The angular scattering characteristics of a single red blood cell are represented better by the Gegenbauer kernel phase function (Reynolds and McCormick 1980) than by the Henyey–Greenstein phase function (Henyey and Greenstein 1941), as was shown by Yaroslavsky *et al* (1996) and by Hammer *et al* (1998) from single scattering experiments with erythrocytes. Thus the Gegenbauer kernel phase function was used for the simulations:

$$p(\mu) = \frac{\alpha g}{\pi W_0} \frac{(1-g^2)^{2\alpha}}{[(1+g)^{2\alpha} - (1-g^{2\alpha})](1+g^2-2g\mu)^{(1+\alpha)}} \tag{2}$$

where $|g| \leqslant 1$, $\alpha > -\frac{1}{2}$, W_0 is the albedo and μ is the cosine of the scattering angle. The absorption and the scattering coefficients were calculated from absorption and scattering cross sections given by Hammer *et al* (1998) using equations (1) as $\mu_a = 22.7\ \text{mm}^{-1}$ and $\mu_s = 221\ \text{mm}^{-1}$. The parameters g and α were varied until the simulated angular distribution of scattered light power fitted the measured one.

3. Results and discussion

The measured as well as the simulated light powers versus scattering angle are represented in figure 1. The best agreement between the measured and the calculated data was achieved with $g = 0.972$ and $\alpha = 0.49$. The Gegenbauer kernel function is a generalization of the

Henyey–Greenstein function, and can be reduced to the latter by setting $\alpha = 0.5$. Therefore the Henyey–Greenstein function with $g = 0.972$ is suitable as the effective phase function for the description of light scattering in blood with physiological haematocrit at the wavelength of 514 nm. This is an important finding since the Henyey–Greenstein phase function is a simple and convenient approximation, which is widely used in radiation transport calculations because of its mathematical tractability. However, the results of the research show that the scattering anisotropy factor g is remarkably reduced in whole blood compared with that of single erythrocytes, which was shown to be approximately 0.997 by the Gegenbauer kernel phase function as well as by the Mie phase function (Hammer *et al* 1998) for the same wavelength. The demonstrated broadening of the scattering characteristics of whole blood compared with those of single red blood cells is regarded to be an effect of the interference of waves scattered at neighbouring cells.

The difference between the anisotropy factor of 0.972 and 0.997 is not large. At the same time, the reduced scattering coefficient $\mu_s' = (1 - g)\mu_s$, often used in radiation transport calculations, differs between 6.188 mm^{-1} and 0.663 mm^{-1} ($\mu_s = 221\ \text{mm}^{-1}$), which is a huge difference. The results of the study show that the Henyey–Greenstein phase function with an anisotropy factor of 0.972 is a valid approximation for the effective scattering phase function of blood with physiological haematocrit at the wavelength of 514 nm. The good agreement obtained between the measured goniophotometric data and the simulated angular distribution of scattered light prove that the Henyey–Greenstein phase function should be used for radiative transport calculations in non-diluted blood.

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