

# Experimental and Theoretical Study of Light Scattering by Individual Mature Red Blood Cells with Scanning Flow Cytometry and Discrete Dipole Approximation

Maxim A. Yurkin<sup>1,3</sup>

Konstantin A. Semyanov<sup>2</sup>

Alfons G. Hoekstra<sup>1</sup>

Valeri P. Maltsev<sup>2</sup>

1: Faculty of Science, Section Computational Science, of the University of Amsterdam, Kruislaan 403, 1098 SJ, Amsterdam, The Netherlands, tel: +31-20-525-7530, fax: +31-20-525-7490

2: Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, Institutskaya 3, Novosibirsk 630090 Russia, tel: +7-3832-333240, fax: +7-3832-342350

3: email: myurkin@science.uva.nl

## Abstract

Elastic light scattering by mature Red Blood Cells (RBC) was theoretically and experimentally analyzed with the Discrete Dipole Approximation (DDA) and the Scanning Flow Cytometry (SFC) technique, respectively. The SFC allows measurement of angular dependence of light-scattering intensity (indicatrix) of single particles. A mature RBC is modeled as a biconcave disk in DDA simulations of light scattering. We have studied the effect of RBC orientation relating to the direction of the incident light on the indicatrix. Numerical calculations of indicatrices for different axis ratios and volumes of RBC have been carried out. Comparison of the simulated indicatrices and indicatrices measured with the SFC showed good agreement, validating the biconcave disk model for a mature RBC. We simulated the light-scattering output signals from the SFC with DDA for a RBC modeled as a disk-sphere and an oblate spheroid. The biconcave disk, disk-sphere, and oblate spheroid models have been compared for two orientations relative to direction of incident beam, face-on and rim-on incidence. Only the oblate spheroid model for rim-on incidence gives results similar to the rigorous biconcave disk model.

## 1 Introduction

In the area of medical diagnostics understanding how a laser beam interacts with blood suspensions or a whole-blood medium is of paramount importance in quantifying the inspection process in many commercial devices and experimental setups that are used widely for *in vivo* or *in vitro* blood measurements. These measurements are mainly focused on electromagnetic scattering properties of Red Blood Cells (RBC), which are most numerous in the blood. Light scattering properties of the suspension are based on a solution of the single-electromagnetic-scattering problem for a RBC.

A well-known method to simulate light scattering by arbitrary shaped particles is the Discrete Dipole Approximation (DDA) [1]. The latest improvements of DDA and its implementation on parallel super-computers [2] allow simulation of light scattering by particles with the size of RBCs. The next generation of flow cytometers, the Scanning Flow Cytometer (SFC) [3], allows measurement of angular dependence of light-scattering intensity (indicatrix) of single particles at a speed of  $O(10^2)$  particles per second.

In this paper, light scattering by mature RBCs was theoretically and experimentally analyzed with DDA and SFC, respectively. In the numerical simulations we modeled a mature RBC as a biconcave disk [4].

## 2 Theory

A mature red blood cell (RBC) can be modeled as a biconcave discoid. A RBC is composed of hemoglobin (32%), water (65%), and membrane components (3%) and does not contain any nucleus. The shape of the RBC is described by Fung *et al* [4], we have rewritten it in the following form

$$T(x) = \varepsilon d \sqrt{1 - x^2} (0.1583 + 1.5262x^2 - 0.8579x^4), \quad (1)$$

where  $T$  is a thickness of RBC (along the axis of symmetry),  $x$  is a relative radial cylindrical coordinate  $x = 2\rho/d$  ( $-1 \leq x \leq 1$ ),  $\rho$  – radial cylindrical coordinate,  $d$  – diameter of the RBC,  $\varepsilon = T_{\max}/d$  is an aspect ratio of maximum thickness and diameter.

We used the code by Hoekstra *et al* [2] for DDA computations, which runs very efficiently on distributed memory computers, provided that the number of dipoles per processor is large enough. The size of the dipoles was varied in the range  $\lambda/11 - \lambda/8$  for different sizes of RBCs because of requirements for lattice regularity in the current implementation of the parallel FFT algorithm [2].

## 3 Experimental equipment and procedures

The experimental part of this study was carried out by means of the Scanning Flow Cytometer (SFC) that allows measurement of the angular dependency of light-scattering intensity in the region ranging from  $5^\circ$  to  $100^\circ$ . The design and basic principles of the SFC were described in detail elsewhere [3]. The current set-up of the SFC provides measurement of the following combination of Mueller matrix elements:

$$I_s(\theta) = \int_0^{2\pi} [S_{11}(\theta, \varphi) + S_{14}(\theta, \varphi)] d\varphi, \quad (2)$$

where  $I_s(\theta)$  is the output signal of the SFC,  $\theta$  and  $\varphi$  are the polar and azimuthal angles, respectively. After integration over azimuthal angle  $\varphi$  the second term in Eq. (2) vanishes, because of the axisymmetry of RBCs. Therefore, the SFC output signal will be proportional to  $S_{11}$  integrated over azimuthal angle. In order to compare the experimental and theoretical light scattering from RBCs we used the DDA for calculation of the indicatrices. Our SFC setup allowed reliable measurements in the angular range  $10^\circ - 50^\circ$  (because of the operational range of the analog-digital converter).

A sample containing approximately  $10^6$  cells per ml was prepared from fresh blood using buffered saline for dilution. We continuously measured 3000 indicatrices of RBCs with the SFC. Each of them was compared with each of the calculated theoretical indicatrices by calculating a  $\chi^2$ -distance with a weighting function:

$$w(\theta) = \sin^2\left(\pi \frac{\theta - 10^\circ}{50^\circ - 10^\circ}\right), \quad (3)$$

which is intended to suppress experimental errors. An experimentally measured RBC is said to have characteristics of the closest (by  $\chi^2$ ) theoretical indicatrix, if their  $\chi^2$ -distance is less than a threshold. The threshold was set empirically to 40.

The optical model of the RBC was used in calculations with the profile described by Eq. (1). The RBC's diameter was varied from  $6 \mu\text{m}$  to  $9 \mu\text{m}$ . The wavelength  $\lambda$  was  $0.6328 \mu\text{m}$ . The refractive index of the surrounding medium (saline) was 1.333. The RBC's refractive index was fixed at 1.40 (with negligible imaginary part) that results to relative refractive index of 1.05.

## 4 Results and discussion

We have made calculations of indicatrices for several biconcave disks with different diameters and volumes filling a small database to be used in the inverse problem. For each set of diameter and

volume four indicatrices were calculated for orientation angles  $\beta$  (between the axis of the biconcave disk symmetry and the direction of the incident beam) –  $60^\circ$ ,  $70^\circ$ ,  $80^\circ$  and  $90^\circ$ .

In order to provide an effective comparison of experimental and theoretical light-scattering data the indicatrices were modified by multiplication with the weighting function  $w(\theta)$  (Eq. (3)). A few representative results of comparison of experimental and theoretical modified indicatrices are presented in Fig.1. One can see that theoretical indicatrices fit experimental curves well. All the experimental indicatrices which passed a  $\chi^2$ -threshold were used to plot a distribution of mature RBCs over the orientation angle, as presented in Fig.2. This distribution proves that orthogonal orientation is much more preferable for RBCs in the capillary of SFC which agrees with our previous results.

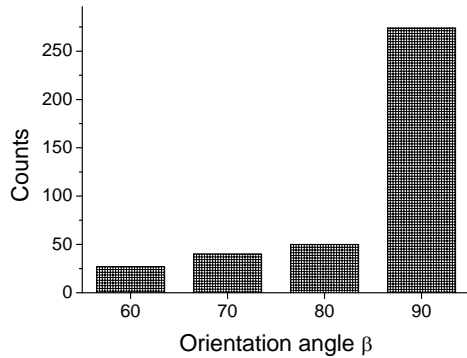


Fig. 2. Distribution of mature RBCs over orientation angle obtained using  $\chi^2$ -test.

T-matrix method is much faster but cannot be applied directly to the biconcave disk shape. However, it can be effectively applied to a particle with disk-sphere or oblate spheroid shape, which can be used to model the red blood cells. Therefore we have compared the indicatrix of particles shaped according to Eq. (1) and particles with the following shape geometry: disk-sphere and oblate spheroid. The comparison was performed for the diameter-volume-equal particles. The parameters of the biconcave disk were as follows: diameter  $d = 7.60 \mu\text{m}$ , aspect ratio  $\varepsilon = 0.380$  (volume  $V = 100 \mu\text{m}^3$ ), and relative refractive index  $m = 1.05$ . The light scattering of two orientations of the particles relating to direction of the incident beam, rim-on and face-on incidence, was computed.

The indicatrices of the biconcave disk and disk-sphere differed significantly for both orientations (data not shown). However, the

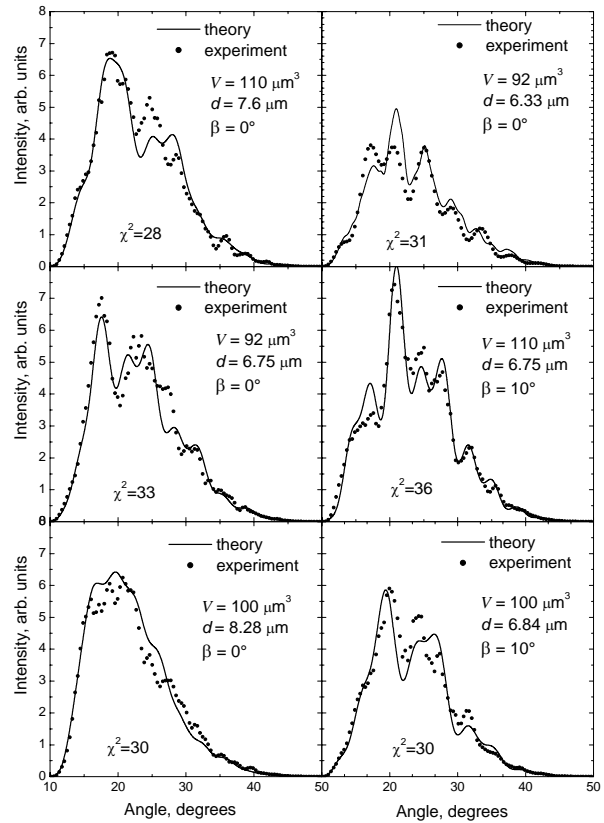


Fig. 1. Experimental and theoretical modified indicatrices of individual mature RBCs. Values of  $\chi^2$  differences are shown on the plots.

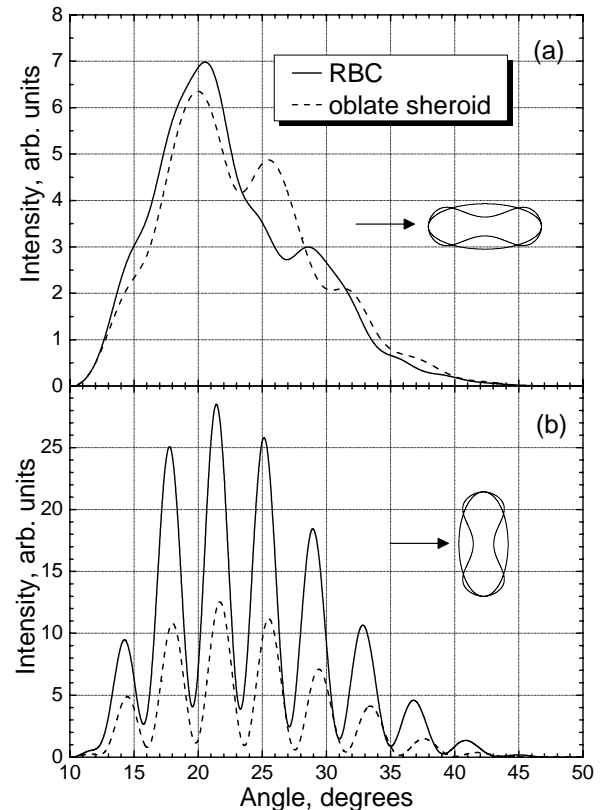


Fig. 3. The modified indicatrices of the biconcave disk and diameter-volume-equivalent oblate spheroid: (a) rim-on and (b) face-on incidence.

spheroid model showed agreement with the biconcave disk model over a wide angular interval, but only for rim-on incidence (Fig. 3). This conclusion is in agreement with the boundary-element methodology applied to study of light scattering of red blood cell. Still the validity of such substitution for calculation of RBC indicatrices should be further studied for different RBC sizes with respect to the certain problem, where these indicatrices are to be used.

## 5 Conclusion

Our simulation of light scattering of a mature RBC has shown that the indicatrix is sensitive to RBC shape and DDA (or some other method assuming no simplifications on RBC shape) should be used in a study of formation of the indicatrix under variation of RBC characteristics. However, our results suggest that light scattering of the RBCs can be simulated with the T-matrix method for the rim-on incidence by using the oblate spheroid model. Fortunately the hydrodynamic system of the SFC delivers mature RBCs into the testing zone in this specific orientation. This performance of the SFC gives a chance of solving the inverse light-scattering problem for mature RBCs e.g. by parameterization or a neural network because T-matrix simulation requires substantially less computing time in comparison with the DDA algorithm. However, the precision of such algorithms when developed should be tested using realistic indicatrices obtained e.g. by DDA simulations.

Therefore, need for improvement of current DDA code arises. Such an improvement can not only provide enough testing indicatrices for above described problem but also make feasible such tasks as solving inverse light-scattering problem for any orientation of RBC relative to incident beam.

## Acknowledgement

This research was supported by Russian Foundation for Basic Research through the grant 02-02-08120-inno and 03-04-48852-a, by Siberian Branch of the Russian Academy of Sciences through the grant 115-2003-03-06, and by the NATO Science for Peace program through grant SfP 977976.

## References

- [1] B. T. Draine, P. J. Flatau, "Discrete-Dipole Approximation for Scattering Calculations," *J. Opt. Soc. Am. A* 11, 1491-1499 (1994).
- [2] A. G. Hoekstra, M. D. Grimminck, P. M. A. Sloot, "Large scale simulation of elastic light scattering by a fast discrete dipole approximation," *Int. J. Mod. Phys. C* 9, 87-102 (1998).
- [3] V. P. Maltsev, "Scanning flow cytometry for individual particle analysis," *Rev. Sci. Instruments* 71, 243-255 (2000).
- [4] Y. C. Fung, W. C. Tsang, P. Patitucci, "High-resolution data on the geometry of red blood cells," *Biorheology* 18, 369-385 (1981).