

LIGHT SCATTERING FROM SINGLE AND ORIENTED RED BLOOD CELLS: SENSITIVITY ANALYSIS

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Abstract – We investigate light scattering from single, randomly or actively orientated red blood cells (RBCs) employing a dedicated flow cytometer. The measured bimodal distributions of cross sections of randomly oriented RBCs can be reproduced numerically applying the discrete dipole approximation (DDA). Our sensitivity analysis of the scattering pattern reveals a significant impact of assumed biconcave shapes and different orientations of the hydrodynamically focused RBCs.

Keywords: flow cytometry, red blood cells (RBCs), discrete dipole approximation (DDA), sensitivity analysis

1. BACKGROUND

Light scattering of laser beams is a widely used tool in flow cytometry to distinguish between various cell populations [1]. We have measured forward light scattering from single oriented erythrocytes in a flow cytometer at wavelengths between $\lambda=379.5$ nm and $\lambda=632.8$ nm. In histograms accumulated for up to 300,000 erythrocytes bimodal distributions of the integrated scattering cross section were observed (cf. Fig. 1.). To correlate the bimodal distributions and the erythrocytes' alignment we have constrained the orientations of the scattering erythrocytes by modifying the injection geometry of the sample flow in the sheath flow, used for hydrodynamic focussing. The measured light diffraction patterns are compared with numerical calculations based on the discrete dipole approximation.

2. METHODS

A home-made laser flow cytometer was used to measure the absolute small angle forward scattering cross sections for native and sphered erythrocytes at different wavelengths. We investigated the dependence of the integrated small angle scattering signal on the solid angle of observation. To validate our interpretation of the bimodal distribution of integrated cross sections, discrimination between different orientations of erythrocytes in the flow channel was achieved by actively aligning the erythrocytes with a slit-shaped injection needle. Injection of the sample flow in the sheath flow

resulted in an alignment of erythrocytes with their long axis parallel to the slit.

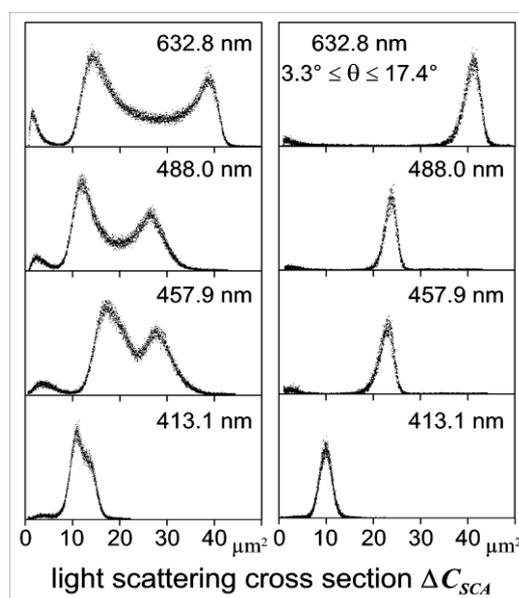


Fig. 1. Frequency distributions of measured cross sections of native (left column) and sphered (right column) erythrocytes at different wavelengths.

For comparison with numerical calculations we compute the amplitude matrix and the Mueller matrix of the scattered light for different wavelengths of the incident light applying the discrete dipole approximation (DDA). For these simulations we used the open source software package ADDA 1.2 [2]. For different ranges of the polar angle θ and the azimuth angle Φ , the Mueller matrix elements were calculated in dependence on different particle orientations relative to the direction of propagation of the incident light. Fig. 2 shows an example of the biconcave RBC profile used in our calculations and sensitivity analysis. For comparison, the half-hemisphere and the corresponding cross sections are depicted, too, in the lower part of the upper diagram and in the lower diagram in Fig. 2 (red symbols).

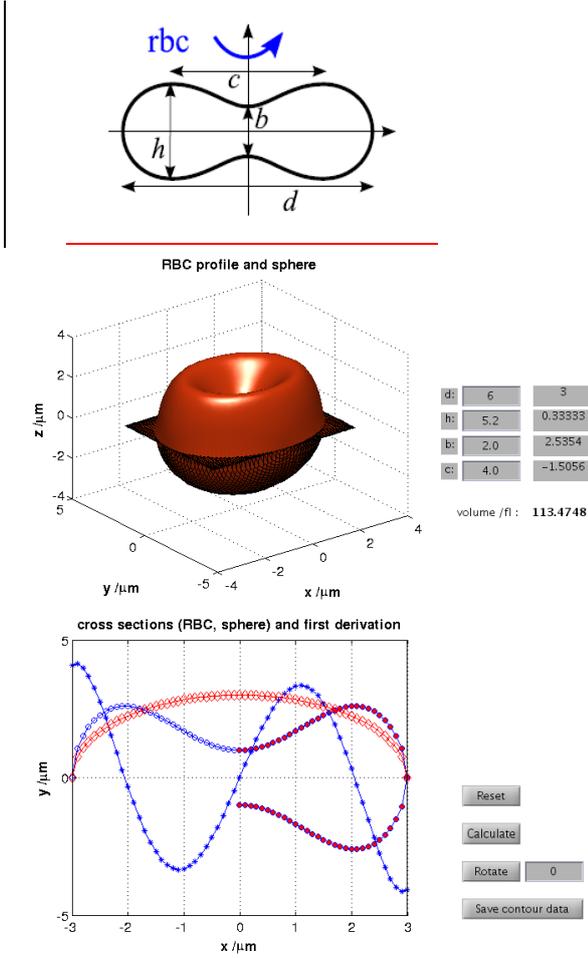


Fig. 2. Example of the biconcave RBC profile model with a diameter of 6 μm and a volume of 113.47 μm^3 used for our calculations.

3. RESULTS

Integrated scattering cross sections vary up to a factor of three for different orientations of the red blood cells, sensitively depending on the angle of observation and the wavelength used for irradiation. The active RBC orientation led to a suppression of one of the peaks of the measured bimodal distribution of the scattering cross section. The supplementing analysis applying different DDA simulation scenes in dependence on the RBC orientation and a biconcave profile of the red blood cells are supporting the experimental findings.

DDA numerical simulations of the light scattering of RBCs with different shapes are feasible and very sensitive. The RBC particle orientation reveals a significant impact on the Mueller matrix, in particular on its element $S_{11}(\theta, \Phi)$. The impact is valid for the scattered light intensities, too, as demonstrated by evaluating for example the squared scattering amplitude

$$S_1^2(\theta, \Phi) = 0.5 (S_{11}(\theta, \Phi) - S_{12}(\theta, \Phi) - S_{21}(\theta, \Phi) + S_{22}(\theta, \Phi))$$

or the linear combination

$$S_{11}(\theta, \Phi) + S_{12}(\theta, \Phi) \cos(2\Phi) + S_{13}(\theta, \Phi) \sin(2\Phi)$$

which describes measured light intensity distributions [3], provided geometrical and optical properties of the particles are accounted for in the calculation.

Furthermore, the sensitivity analysis reveals that also for the lateral scattering directions, i.e., a range from 88° to 92° for the polar angle θ , the differential scattering cross sections are significantly affected by the particle orientation for the biconcave RBC profile used in our calculations. Fig. 3 shows an example for the intensity distributions for two different RBC particle orientations around the flow axis of the cytometer, according to the last equation.

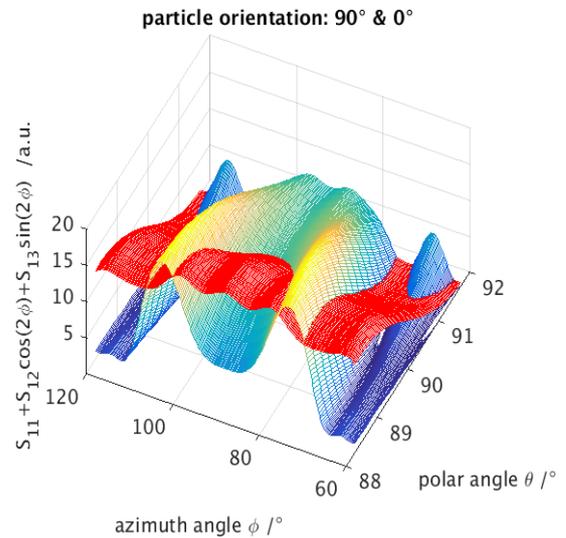


Fig. 3. Example of lateral light intensity distributions for two different orientations around the flow axis (results for particle orientation of 0° in red color); biconcave profile of the RBC used for the calculation with a diameter of 6 μm and a volume of 113.47 μm^3 .

4. CONCLUSIONS

In order to interpret the measured bimodal distributions of the integrated scattering cross section of randomly oriented RBCs a detailed sensitivity analysis was executed. We have applied the discrete dipole approximation model to calculate the Mueller matrix and derived differential cross sections in dependence on different shapes and orientations of the red blood cells.

Our results reveal a high sensitivity with respect to orientation and shape. Particularly, a biconcave profile model of the RBC seems to be a good starting

point for evaluating measured light diffraction patterns of single RBCs. The reconstruction of the volume of a single RBC including the orientation impact seems to be possible. It is expected that the uncertainty of the reconstructed particle volume can be reduced significantly when taking orientation into account. Further investigations will focus on this issue.

REFERENCES

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