Aggregates of biological cells: surprising features in light scattering

A. E. Moskalensky, M.A. Yurkin, V.P. Maltsev

Voevodsky Institute of Chemical Kinetics and Combustion SB RAS, Institutskaya 3, Novosibirsk, 630090, Russia;
Novosibirsk State University, Pirogova 2, Novosibirsk, 630090, Russia.

Biospecific aggregation is a common process in biology. For instance, aggregation of blood platelets is a key process in hemostasis. Optical methods, including light-transmission aggregometry and flow cytometry, are widely used to quantify the aggregation. This work is devoted to the features of light scattering by aggregates of biological cells. Since typical sizes of cells are of order of several wavelengths, light scattering by individual cells and their aggregates needs to be computed using a rigorous numerical approach such as T-matrix or DDA. We performed such simulations systematically for a large number of different aggregates and found surprising phenomena. Light scattering patterns of aggregates averaged over the azimuthal angle are approximately equal to sum of that of monomers, if the latter do not shadow each other. These phenomena were also observed experimentally with the scanning flow cytometry.

INTRODUCTION

The adhesion of blood platelet to endothelial cells or other platelets play a central role in hemostasis. The latter is referred to as platelet aggregation. It occurs normally in response to vessel wall injury or other physiological stimuli [1]. Platelet aggregation is very dangerous when become inappropriate, i.e., when platelets aggregate spontaneously or due to low dose of agonist. On the other hand, platelets are easy to obtain and cause an aggregation *in vitro*, which make them a model object for studying aggregation of biological cells.

The "gold standard" in clinical practice for the assessment of platelets aggregation is light-transmission aggregometry, or turbidimetry. It is also used for immunoagglutination test, where biospecific aggregation of polystyrene beads depends on the concentration of a specific protein in a serum. The disadvantage of this technique is the inability to trace the early stage of aggregation: the light transmission of suspension changes markedly only when large proportion of particles formed aggregates.

The flow cytometry is an instrument measuring light scattering and fluorescence of individual particles in a flow. It is also used to study platelet function. For example, the platelet-leukocyte aggregates can be identified with the flow cytometry using fluorescent labels [2]. Unfortunately, platelet-platelet aggregates cannot be identified in a similar manner due to heterogeneity of cells. This problem may be solved with the scanning flow cytometer which measures a light-scattering profile (LSP) of each particle [3]:

$$I(\theta) = \frac{1}{2\pi}\int_0^{2\pi}\left(S_{11}(\theta,\varphi) + S_{14}(\theta,\varphi)\right)d\varphi\,, \qquad (1)$$

where S is the Mueller matrix, and θ and φ are polar and azimuth scattering angles. Our goal is to use this information to distinguish individual platelet from aggregates. Thus, it is important to understand how $I(\theta)$ differs for these two types of particles.

NUMERICAL SIMULATION FOR AGGREGATES OF BLOOD PLATELETS

We simulated aggregates of blood platelets based on our previous work [4]. Each platelet can be modeled by a homogeneous oblate spheroid, which has 5 parameters with respect to light scattering simulation: two sizes, refractive index and two Euler angles. The first Euler angle is irrelevant in our case due to axial symmetry of Eq. (1).

As the first step, we simulated LSPs of aggregates of two platelets, which are formed at the very first stage of platelet aggregation. Two oblate spheroids corresponding to activated platelets were specified by four parameters each. These parameters were randomly chosen from physiological ranges for activated platelets. Then a random rotation around the z-axis was given to the second spheroids. Each spheroid was then discretized into a set of dipoles so that 12 dipoles fall on the wavelength or the thickness of spheroid if the latter was smaller. The second spheroid was being moved by one dipole along the positive direction of the z-axis, until two sets of dipoles stopped overlapping. A resulting dipoles configuration was saved to a file, and light scattering was calculated with ADDA v. 1.1 [5]. A typical configuration is shown in Figure 1. During the calculation, Euler angle β for the dimer was again randomly picked out. Total 8756 LSPs of dimers and its constituent spheroids were calculated.

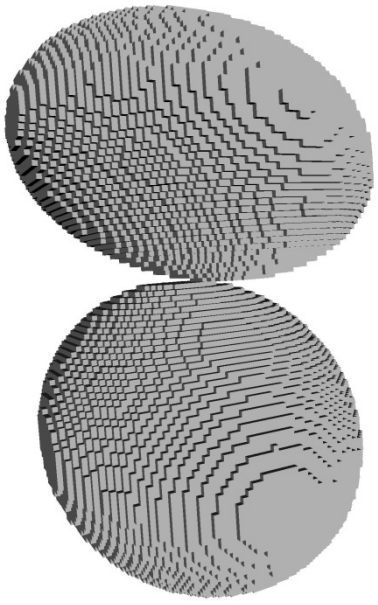

Figure 1. A constructed set of dipoles describing the geometry of dimer of blood platelets. Visualized with LiteBil.

It was surprising that the mean LSPs for dimers and the mean LSP for single platelets are almost identical except for overall intensity and perhaps forward scattering (0-5 degrees). Namely, the mean LSP of dimer appeared to be the doubled mean LSP of single spheroid. The same fact was observed if one replaces all the LSPs of spheroids by independently calculated set. We used previously made database of spheroids LSPs [4] to test this statement.

We also compared each calculated LSP for dimers with the sum of its constituents' LSPs. In many cases the agreement was quite good, mostly when two spheroids were not shadowing each other with respect to the incident wave (e.g., see Figure 2). However, two-dimensional LSPs (i.e., before the integration over azimuthal angle in Eq. (1)) are completely different in all cases. LSPs of individual spheroids have a smooth dependence on the angle φ,

while LSPs of aggregates are highly oscillatory versus it (

Figure 3). This means that integration over azimuthal angle destroys the structure responsible for interaction of particles in dimer. It can be explained considering the phase difference of fields scattered by each particle in the case when no multiple scattering present (each monomer is treated without any approximations). This approximation is surprisingly valid in many cases, depending on orientation of dimer, which we also tested by the examination of internal fields of particles. The internal fields were unchanged even when one particle shadows a significant part of another, which may be related to a small refractive index of blood platelets (~1.03 relative to the water).

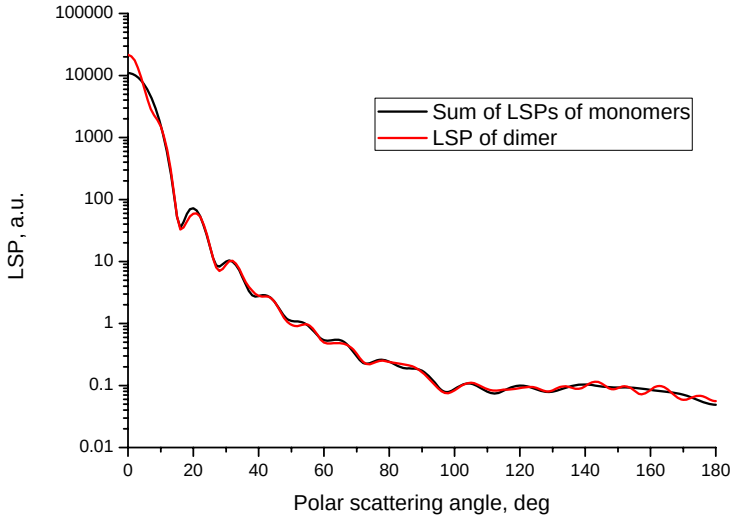

Figure 2. Light scattering patterns of dimer and sum of LSPs of its monomers.

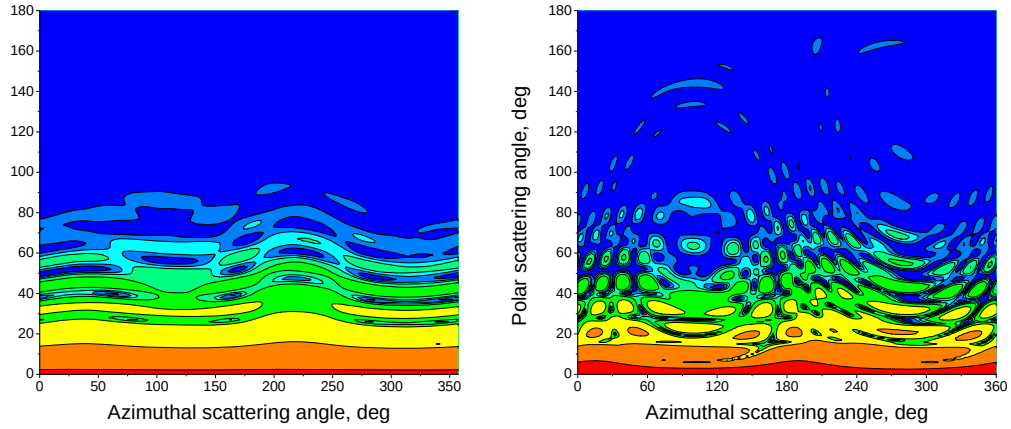

Figure 3. Two-dimensional light-scattering profiles for dimer of blood platelets (on the right) and for the sum of LSPs of its monomers (on the left).

EXPERIMENT AND SIMULATIONS FOR POLYSTYRENE BEADS

Polystyrene beads are widely used in flow cytometry for device alignment and calibration. They are available with different sizes and characterized by spherical shape and narrow size distribution. In normal conditions, they could spontaneously make small aggregates. FITC- (or another dye-) labeled beads allows one to determine the size of aggregate since fluorescence signal is directly proportional to number of beads.

We measured FITC-labeled beads and identified single particles and dimers. Some LSPs of dimers were the doubled LSPs of monomer. This is the same effect that we observed in simulation of blood platelets dimers.

Polystyrene beads have two major differences from blood platelets: they are spherical and have larger refractive index (1.2 relative to the water). Therefore, we conducted the simulations for dimers of beads with the MSTM code [6] and found the same oscillatory structure versus the azimuthal angle. However, the LSP changed faster with the shadowing of one particle by another.

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