Application of the scanning flow cytometry for characterization of blood platelets

Alexander E. Moskalensky[a,b,c], Maxim A. Yurkin[a,b], Anastasiya I. Konokhova[a,b], Dmitry I. Strokotov[a,b], and Valeri P. Maltsev[a,b]

[a]*Institute of Chemical Kinetics and Combustion SB RAS, Institutskaya Str. 3, 630090, Novosibirsk, Russia*
[b]*Novosibirsk State University, Pirogova Str. 2, 630090, Novosibirsk, Russia*
[c]*sunmosk@mail.ru*
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Scanning flow cytometer (SFC) is capable of measuring angle-resolved light-scattering pattern (LSP) of individual particles in flow. Specifically, the LSP is an intensity of scattered light integrated over the azimuthal scattering angle as a function of the polar scattering angle. An analysis of the LSP potentially allows one to determine morphological characteristics of biological particles. However, such characterization constitutes the inverse light-scattering problem. This work is dedicated to characterization of blood platelets from LSPs measured with the SFC.

Blood platelets are small disc-shaped cells without nuclei. Their main function is the formation of hemostatic plugs after vessel wall injury. This is achieved by platelet activation in response to the injury, followed by platelet aggregation and adhesion to the damaged area. Activated platelet rapidly changes its shape from discoid to more spherical with numerous pseudopodia. The study of platelets morphology, activation and aggregation are of clinical importance.

We use an oblate spheroid as an optical model of a blood platelet. Since pseudopodia were shown to slightly affect the LSP, activated platelets were also modeled as oblate spheroids. This model has four parameters which are to be obtained by solving the inverse light scattering problem: refractive index, two dimensions and one orientation angle. Theoretical LSPs for oblate spheroids were calculated using the discrete dipole approximation (DDA). In particular, we used open-source code ADDA v.1.0. Calculation of one LSP took approximately 1 min on a single core. The computations were verified by comparison with other light-scattering codes. We also performed simulations for dimers of platelets to effectively separate them from single cells based on LSPs.

Given the optical model, the inverse light scattering problem is transformed to global optimization, minimizing the discrepancy between experimental and theoretical LSPs. Direct fitting is unfeasible due to large computational time, therefore a pre-calculated set of LSPs is used to perform global optimization by nearest-neighbor interpolation. The best estimate of parameters, mathematical expectations and covariance matrix are determined for each experimental LSP. This general approach is applicable not only for blood platelets but also for any particles with optical model described by a few parameters.

The approach was applied to characterize blood platelets from healthy donors. LSPs of resting platelets and ones stimulated by several agonists were measured with the SFC. The activation state of platelets additionally monitored using activation-dependent fluorescent label showed a correlation with platelets shape obtained with our approach. The results of global optimization are also in good agreement with microscopic measurements and resistive-particle counting. A detailed account of some of these results can be found in the paper [1].

[1] Moskalensky AE, Yurkin MA, Konokhova AI, Strokotov DI, Nekrasov VM, Chernyshev AV, Tsvetovskaya GA, Chikova ED, and Maltsev VP. Accurate measurement of volume and shape of resting and activated blood platelets from light scattering. *Accepted to Journal of Biomedical Optics*