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Identification of blood platelets aggregates with angle-resolved light scattering in backward hemisphere

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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. The study of platelets morphology, activation and aggregation are of clinical importance.

The scanning flow cytometer (SFC) allows one to measure the light scattering pattern (LSPs) of individual cells. LSPs are the intensity of scattered light integrated over the azimuthal scattering angle *versus* the polar scattering angle from 10º to 70º. We used the SFC to study the aggregation of blood platelets, which implies the identification of monomers and aggregates from light scattering. However, the LSPs of blood platelets aggregates have the same structure as that of individual cells, which makes it impossible to separate monomers from aggregates.

We made a numerical simulation to account for this phenomenon. Oblate spheroid was used as an optical model of a blood platelet. Theoretical LSPs for oblate spheroids and their aggregates were calculated using the discrete dipole approximation (DDA). The simulation showed that single-scattering approximation is valid for the considered aggregates, because they are optically soft ($m \sim$1.03). The integration over the azimuthal scattering angle leads to the vanishing of the interference between monomers, resulting in the additivity of their LSPs.

Due to variability of platelets size and refractive index, one large and/or dense platelet may have LSP exactly as dimer of two smaller cells. This marks the boundary of the applicability of LSP. However, numerical simulation also showed that LSPs of monomer and dimer, being identical in the forward hemisphere, differ significantly in the backward hemisphere. Therefore, measuring LSPs for polar angles 110º-170º is essential for the identification of blood platelets dimer and larger aggregates.

This experimental challenge implies the upgrade of the SFC, consisting in the change of direction of incident laser beam. The sensitivity issue may complicate the measurement due to smaller scattering intensity in backward hemisphere. However, the modernized version of the SFC is currently being manufactured. It should be capable of the measurement of LSPs in the forward and backward angle ranges simultaneously, utilizing two lasers with close wavelengths and the dichroic mirror.