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Characterization of Native Red Blood Cell Morphology by Scanning Flow Cytometry

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We study the capabilities of the Scanning Flow Cytometer (SFC) [1] to determine the geometry and hemoglobin concentration of native Red Blood Cells (RBC). Our previous work in this direction was limited to a single shape model and fixed hemoglobin concentration [2]. Here we remove these limitations. Generalizing different known models for the bi-concave shape of the red blood cell we propose a new model based on 4 parameters: diameter, maximum and minimum thickness and diameter at maximum thickness. Other geometrical parameters, such as volume and sphericity index, can be derived from these four. The SFC measures angle resolved Light Scattering Patterns (LSP) of individual particles [1]. We computed LSPs of RBC over a range of geometrical parameters, hemoglobin concentration (i.e. refractive index) and orientation in the flow of SFC, using our Discrete Dipole Approximation code [3]. In total 40 000 LSPs were computed. We measured some 3 000 LSPs of RBC from a single blood sample with the SFC. Each measured LSP was compared to each theoretical LSP from the database, and a root mean square difference was obtained. We assumed that the best theoretical fit best characterizes the RBC. The volume, hemoglobin concentration, and sphericity index thus obtained correspond to literature data. However, the distributions over these parameters are wider than found in the literature. To explain this fact we have theoretically analyzed the errors of the characterization method and showed that they significantly increase with radius of the flow in SFC. Possibilities to reduce flow radius are discussed.

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[1] V.P. Maltsev and K.A. Semyanov. *Characterisation of Bio-Particles from Light Scattering* (Inverse and Ill-Posed Problems Series, VSP, Utrecht, 2004).
[2] M.A. Yurkin, K.A. Semyanov, P.A. Tarasov, A.V. Chernyshev, A.G. Hoekstra, and V.P. Maltsev. Experimental and theoretical study of light scattering by individual mature red blood cells with scanning flow cytometry and discrete dipole approximation, *Appl. Opt.* **44**, 5249-5256 (2005).
[3] M.A. Yurkin, V.P. Maltsev, A.G. Hoekstra. Capabilities of discrete dipole approximation for simulation of light scattering for particles much larger than the wavelength. *J Quan.t Spectros. Radiat. Transf.* (accepted).