Light Scattering by Granulated Spheres Applied to Discriminate Subtypes of Granulated Blood Cells

M. A. Yurkin,[a,b] K. A. Semyanov,[b] V. P. Maltsev,[b,c] and A. G. Hoekstra[a]
[a] *Section Computational Science, Faculty of Science, University of Amsterdam, Kruislaan 403, 1098 SJ, Amsterdam, The Netherlands*
[b] *Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, Institutskaya Str. 3, 630090, Novosibirsk, Russia*
[c] *Novosibirsk State University, Pirogova Str. 2, 630090, Novosibirsk, Russia*
myurkin@science.uva.nl

Granulated blood cells (granulocytes) constitute about 70% of White Blood Cells (WBC) and can be divided into three subtypes: neutrophils, eosinophils, and basophils. The latter are few in number; hence, we do not consider them further in this report. Typical size of granulocytes in suspension is 8-9 μm; their nucleus consists of a few lobes. They scatter light relatively intensely in the side direction because of granules and complex nucleus. Therefore, they can be easily separated from other WBC on the standard forward vs. side scattering cytometric map [1]. Discrimination between neutrophils and eosinophils can be performed based on the depolarization ratio measured at side direction [2]. It is believed that difference in depolarization ration is caused by the different sizes of granules, which is less than 0.5 and 1.3 μm for neutrophils and eosinophils respectively. However, the effect has never been rigorously explained.

To better understand light scattering properties we used a model particle. A sphere with diameter 8 μm was randomly filled with spherical granules. We varied the diameter of granules from 0 to 2 μm and their volume fraction from 0.02 to 0.3. Light scattering was simulated rigorously using discrete dipole approximation code ADDA v. 0.76 [3] for wavelength 0.66 μm and the refractive index of outer medium 1.337. Using the literature data on the morphology of neutrophils and granulocytes we obtained quantitative agreement with the experimental results described above. We tried different refractive indices of the sphere and granules; however, it did not change the conclusion. We also showed that part but not all of the simulated results can be described by Railegh-Gans-Debye approximation.

Currently we are measuring Light Scattering Patterns (LSP) of granulocytes using the scanning flow cytometer [4]. We will compare them with the simulated ones to see how well our simple model, which do not consider nucleus, describes different features of LSPs (other than depolarization ratio). We will present the conclusion of this comparison at the conference.

---

1. L. W. M. M. Terstappen, D. Johnson, R. A. Mickaels, J. Chen, G. Olds, J. T. Hawkins, M. R. Loken, and J. Levin, "Multidimensional flow cytometric blood-cell differentiation without erythrocyte lysis," Blood Cells **17**, 585-602 (1991).
2. B. G. De Grooth, L. W. Terstappen, G. J. Puppels, and J. Greve, "Light-scattering polarization measurements as a new parameter in flow cytometry," Cytometry **8**, 539-544 (1987).
3. "Amsterdam DDA," http://www.science.uva.nl/research/scs/Software/adda (2006).
4. V. P. Maltsev and K. A. Semyanov, *Characterisation of Bio-Particles from Light Scattering*, (VSP, Utrecht, 2004).