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LIGHT SCATTERING PROPERTIES OF NEUTROPHILS

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ABSTRACT

Neutrophils, a subtype of white blood cells, play a key role in allergic reactions. They play a key role in allergic reaction. For the analysis of blood optical methods have found the main application in flow cytometers, allowing simultaneously to measure signals of light scattering and fluorescence from single cells with a speed up to hundreds thousand cells a minute that provides the fast and qualitative analysis.

The objective of this research is to study both experimentally and theoretically the light-scattering properties of human neutrophils. The experimental measurements were performed with the scanning flow cytometer (SFC), which enables rapid measurement of the light-scattering profile (LSP) from an individual moving particle. The measurable range of scattering angles is from 5^0 to 60^0.

The results of theoretical simulations allow one to approach the problem of neutrophil characterization.

Key words: Neutrophils – allergy - light-scattering profile (LSP).

INTRODUCTION

Allergic diseases are widespread and their occurrence seems to increase in Western society. In children for instance such allergic reactions are mainly located in the skin.

Likewise they are strongly involved in immune reactions and inflammatory processes **[Maltsev.& Semyanov (2004)].**

The morphology, metabolism and the basic functions of neutrophils are at present qualitatively studied, tens various products of their secretion are characterized. Damage of functions of neutrophils leads to various diseases. These defects can be congenital or got as a result of damaging action on neutrophils various factors: bacteria, viruses, medical products, etc. Clinical displays of the majority of defects of neutrophils are infectious defeats of skin and mucous covers. Within last decade essentially new generation of preparations, fill up insufficiency of functions of neutrophils is developed. Many diseases have hematologic displays, for example, some characteristics of blood cells, in particular neutrophils, fall outside the limits physiological norms. Therefore the analysis of blood is the main component of any diagnostic researches. At present optical methods of identification and characterization of blood cells, such as light scattering and fluorescence are widespread **[Maltsev & Semyanov (2004) and Visser (1991)].**

The purposes of the present research were: to measure differential cross-section of neutrophils; to theoretically simulate differential cross-section of neutrophils using a proposed optical model; to compare theoretical and experimental differential cross-sections.

MATERIAL AND METHOD

We have developed an optical model of a neutrophil to compare experimental results with theoretical simulation. Simulation of light scattering of the cell model was performed with the discrete dipole approximation.

The essential feature of the SFC is ability to measure absolute differential light-scattering cross-section of single particles of any form and structure.

In order to determine the differential cross-section for neutrophils we measured the blood leukocytes and polystyrene microbeads (Duke Scientific Corporation, 269C) with a size of 5 μm simultaneously. The neutrophil LSPs were identified from specific fluorescent signal.

This optical model of neutrophil has been used for calculation of differential light-scattering cross-section by the DDA.

With the next stage of the study we have compared experimental LSPs of neutrophils and calculated from the optical model of the cell.

We measured LSPs of neutrophils for four donors to estimate variability of light-scattering for different individuals.

RESULTS

We have observed substantial differences in the measured set of profiles for different donors.

The differential cross-section was calculated from the following equations:

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

Where S_{11} and S_{14} are the scattering matrix elements [**Yurkin, et al., (2005)**]. θ and φ are the polar and azimuth scattering angles, respectively.

Results of measurement of sample of one individual are presented in Fig. 1.

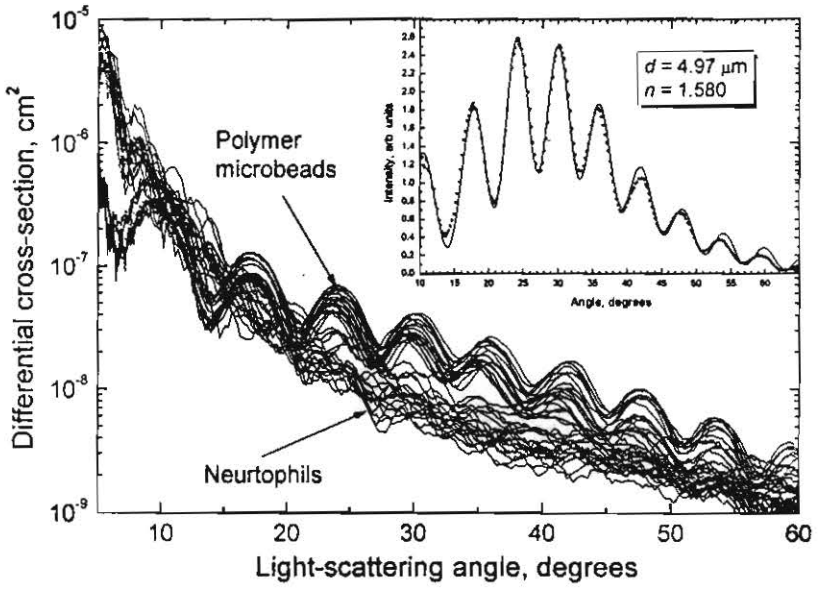

Fig (1): Differential light-scattering cross-section of neutrophils and polymer microbeads. Right-up corner plot shows the experimental (points) and best-fit (solid) light-scattering profiles of one polymer microbead. The best-fit profile corresponds to a sphere with a size of 4.97 μm and refractive index of 1.580.

$$\sigma = \frac{I_s}{(\frac{2\pi n_0}{\lambda})^2}, \qquad (2)$$

Where I_s is the signal from the SFC (Eq.1). λ is the wavelength of the incident light. n_0 is the refractive index of surrounding medium.

Twenty LSPs of neutrophils and microbeads form two well-distinguishable sets of solid curves of individual particles. We pulled out the LSP of the single microbead to find the best-fit LSP calculated from Mie theory.

The fitting result is shown on the plot in the right-up corner in Fig. 1.

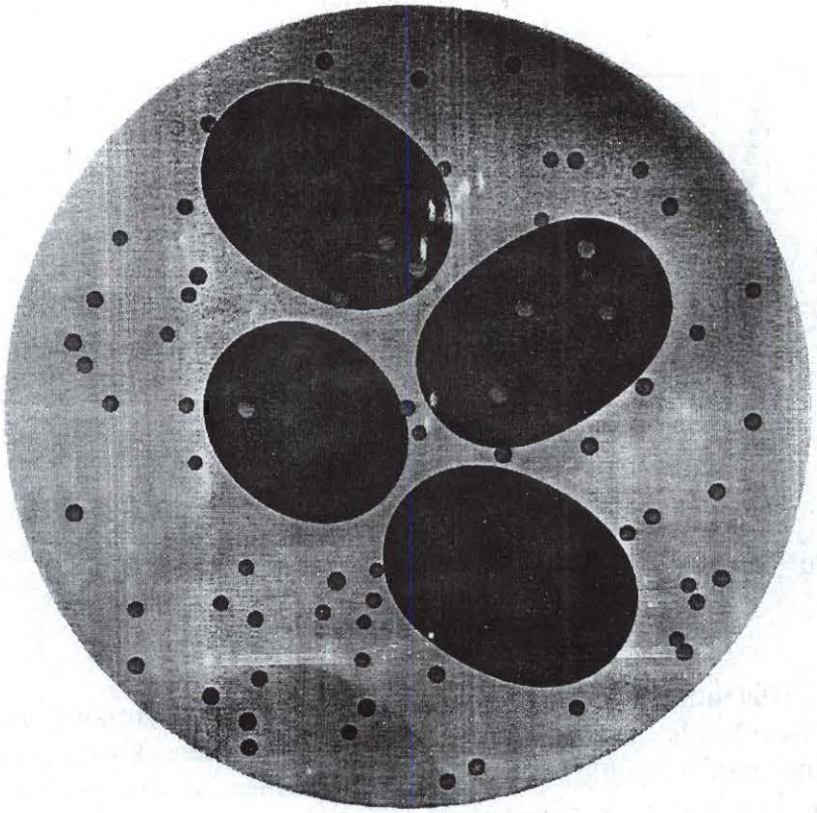

Fig (2): The optical model of a neutrophil

Neutrophils have non-uniform structure and the complex form. As the first approach the optical model of neutrophil in the form of the sphere filled by spheres of smaller diameter - by granules - and a nucleus in the form of four spheroids of the various sizes, which were randomly placed and oriented inside the cytoplasm has been offered (Fig. 2).

Granules were randomly positioned inside the remaining cytoplasm with volume fraction f = 0.1, resulting in total volume fraction of non-cytoplasm material equal to 0.2. The model has following set of parameters which values correspond to literary data on morphology.

Calculation by means of DDA was made for six various sets of the parameters presented in Table 1. We varied both diameter of granules d_g and an angle β rotating the cell relative to the direction of incident laser beam. The results of calculation are presented in Fig. 3.

Table (1):

LSP number	Diameter of granules d_g, μm	Rotation angle β, degrees
N1	0.1	0
N2	0.15	0
N3	0.2	0
N4	0.1	45
N5	0.15	45
N6	0.2	45

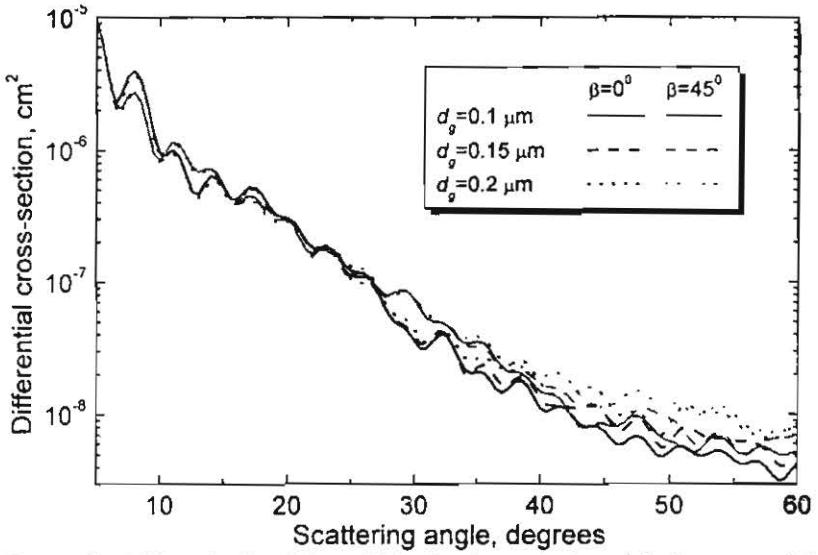

Fig. 3. The LSPs calculated from DDA for the optical model of a neutrophil.

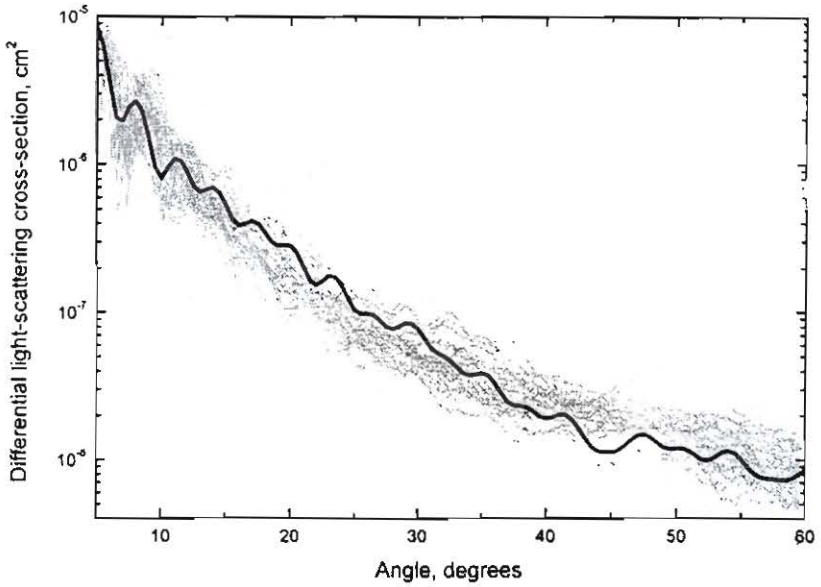

Fig. 4. The experimental LSPs of single neutrophils (gray) and LSP calculated by DDA (black) from the optical model of the cell.

Twenty five randomly chosen experimental LSPs of neutrophils and theoretical LSP calculated for the set N6 (Table 1.) are shown in Fig. 4.

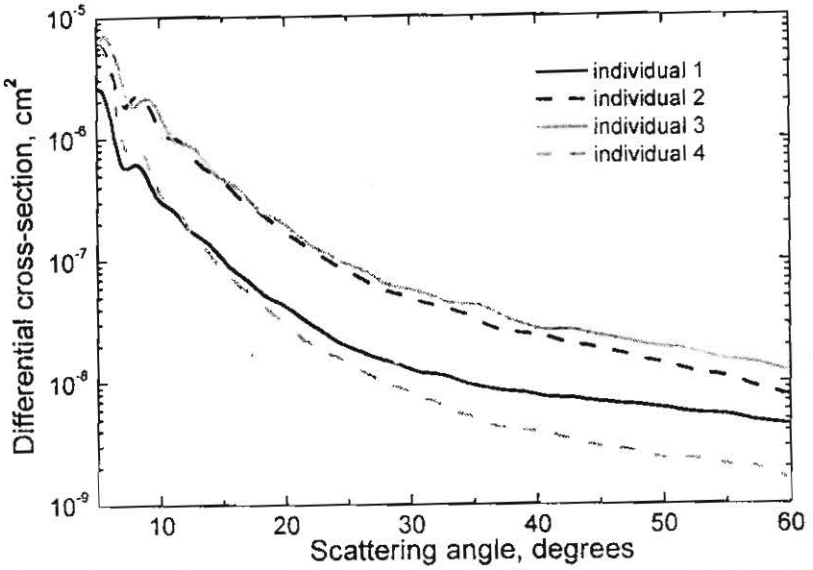

Fig. 5. The experimental LSPs of neutrophils of four donors averaged over sample.

Mean sizes of neutrophils with an error of the mean size and width of distribution (2 standard deviations) are presented in Table 2. There is a small difference in neutrophil sizes between the samples. Moreover, there is an inverse correlation between average size and overall LSP magnitude. Significant changes in neutrophil internal structure (granularity, size of nucleus, refractive indices, etc.) must be involved to explain the five-fold intersample variation of LSP magnitude.

Table (2):

Sample №	Mean diameter, μm	width of distribution, μm
1	9.63±0.04	2.3
2	10.16±0.12	2.6
3	9.50±0.06	4.3
4	10.34±0.16	4.6

DISCUSSION

This work is one of the first in a research of optical properties of neutrophils. These cells represent, apparently, the most complex biological particle. For the first time the efficiency to scatter light by neutrophils was investigated by two the most modern methods of the analysis of single particles, namely, by means of scanning flow cytometry and a method of discrete dipoles.

With this study we have determined the absolute differential cross-section of neutrophils. The measured cross-section can be used in estimation of scattering efficiency of neutrophils in different angular intervals and to estimate a decay of laser radiation by white blood cell sample. The experimental LSPs of neutrophils have demonstrated substation variations in their intensities and structures for individual donor. The variations rise up for neutrophil LSPs of different donors. In order to clarify the reason of these variations we have developed the optical model of a neutrophil that was used in theoretical simulation of light scattering with discrete dipole approximation. The proposed optical model has given a good agreement between experimental and theoretical LSPs. An effect of variations in parameters of the optical model requires future theoretical and experimental studies.

The results of theoretical simulations allow one to approach the problem of neutrophil characterization. However, it does not seem currently feasible to rigorously characterize individual neutrophils. A few simpler ways are possible: either to characterize individual neutrophils using simplifications of the light scattering problem or determine the average morphological parameters from analysis of the averaged LSPs. Even these simplest problems are far from being trivial and still wait to be solved.

The LSP of the polymer microbead measured with the SFC gives perfect agreement with the Mie theory **[Soini *et al.*, (1998)]** that allows marking the plot scale in absolute light-scattering units.

Indeed the experimental points are in a perfect agreement with the solid line that corresponds to a sphere with a size of 4.97 μm and a refractive index of 1.580. The best-fit profile provided the absolute units

for Y-scale in Fig. 1. Differences in differential cross-sections of individual neutrophils are apparently caused by variations in sizes of cells and their internal structures. In order to slightly uncover the reason of these variations we were forced to develop of an optical model of neutrophil and to simulate light scattering with DDA.

In general the absolute cross-section of the LSP calculated from our optical model coincides with experimental LSPs which demonstrate a relatively large variability in their intensities and shapes.

LSPs of individual neutrophils (gray lines Fig. 4) have a random oscillating structure, while averaged LSP is almost featureless except a minimum and maximum between 7° and 10°. These extrema are presented in all individual LSPs as well as in the averaged LSP. The position of extrema of individual LSPs for small scattering angles is mostly determined by neutrophil diameter, i.e. it can be described by diffraction. Averaged LSPs of neutrophils are essentially different we hypothesize that this may serve as a diagnostic parameter. To clarify this, an extensive statistical study in clinics should be carried out.

Finally the experimental plot presents the differential light-scattering cross-section for polymer microbeads and unknown particles.

Further study is required to make any definite conclusions.

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