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OPTICAL PROBING OF THE LYMPHOCYTE TO DETECT INFECTED INDIVIDUALS

Characterization of biological cells or other particles is made by a number of various techniques. Among the most widely used ones is flow cytometry [1]. It is based on single-particle analysis. It tests hundreds or even thousands of particles per second. It is extensively applied for the investigation of normal and pathological leukocytes by combining light scattering and immunophenotyping. The latter is a time-consuming process exploiting expensive monoclonal antibodies. Besides, it uses cell labeling with fluorescence dyes which are potentially toxic or interfere with normal cell functions. Consequently, cell characterization through, mainly, scattering signals is of great interest because of potential reduction of operating costs and speeding up cell characterization as well as minimization or avoiding cell modification with dyes and antibodies. Conventional flow cytometry measuring intensity of scattered light at two directions can be transformed into a much more powerful tool. This end may be achieved by making greater use of the information contained in the scattered light. With this aim in view, one can measure a scattered-light angular distribution. It is far more informative than forward and sideward scattered light and is highly sensitive to different cell characteristics. The idea is implemented for some advanced experimental devices, known as scanning (wide-angle) flow cytometers. The role of the scattered radiation is extended in these instruments. They measure angular dependences of intensity of scattered light ("cell fingerprints") over a wide interval of scattering angles. Such flow-cytometric light-scattering patterns give new opportunities for characterization of normal and pathological cells.

To take advantage of the abovementioned opportunities, one should be able to find a correlation between structures of scattered light and a scatterer. The correlation is obtained by light-scattering theory. It retrieves the parameters of a single particle by solving an inverse light-scattering problem. To solve the inverse problem, optical models of a single scatterer are required. Besides, high rate analysis is very essential for scanning flow cytometry. The recorded angular pattern has to be analyzed rapidly in a real-time scale on the order of milliseconds. That is why it is important to find some means to speed up the computer simulation during the cell characterization. One of the ways to achieve this aim is to develop a cell model as simple as possible in the framework of a particular task. As to the topical task, distinguishing of healthy and infected individuals is of great interest. In the frame of this task, we propose to distinguish healthy and infected individuals on the base of surface structure of peripheral blood lymphocyte. Our viewpoint is derived from the fact that surface state of blood cells is responsible for a large variety of normal and pathological phenomena in an organism of healthy and afflicted persons [2]. Note that lymphocytes actively respond to viruses in blood. For example, the ratio of percentage of smooth and nonsmooth lymphocytes (for instance, folded ones) is noticeably smaller for individuals with persistent viruses of hepatitis C as compared to healthy individuals.

So a potential candidate suitable for fulfilling a role of a prominent structure feature might be hypothetically identified as a surface structure of lymphocytes. Here the following natural question appears, whether this hypothetical distinctive feature of cell morphology could be distinguished by light scattering pattern? If this is the case, then it seems reasonable to pose a task of distinguishing lymphocyte of infected individuals by the inverse light-scattering problem solution.

Having analyzed the morphological, optical and biomededical aspects of the question, we put forward the following assumption: light scattering patterns of the cells with different surface structure features can be used as optical signatures of a normal or pathological status of lymphocyte subpopulations in peripheral blood of the individuals. To test the hypothesis we constructed a simple model of lymphocytes of healthy and infected individuals. Having the model we calculate and analyze the angular patterns of light scattered by the modeling lymphocyte. We present the experimental flow-cytometer data for lymphocytes of healthy and infected individuals to verify the cell model.

We model lymphocytes by two-layer concentric spheres. Non-smooth surface of the cell is modeled as a homogeneous equivolume surface layer. Different thickness ranges of this layer simulate surface features of different kinds, such as, for example, folds and microvilli. The parameters of the cells model in our calculation are obtained on the base of our results [3,4] supplemented with some known data. Since

surface changes of immunocompetent cells under persistent virus infections in question are non-specific [2] we suggest that cell and nucleus sizes and refractive indexes are the same for healthy and afflicted individuals in question.

Before discussion the results of calculation for cell light-scattering patterns, it is worth to note that high-amplitude oscillations of intensity is known to be observed for the spherical cell model, while high-amplitude jumps are not signatures for real cells having some nonsphericity and roughness. To reduce influence of our spherical shape approximation on oscillation pattern of scattered light we average the calculated intensities within a collection angle of seven degrees. Results of the calculations for light scattering patterns of model lymphocytes with non-smooth and smooth surfaces demonstrate that lymphocyte with surface features could be discriminated from smooth lymphocyte by intensity differences. The intensity differences of light scattering patterns for non-smooth and smooth lymphocytes are mostly observed in the backward scattered light. An angles range of scattering for finding the maximum intensity differences to distinguish the cells involved is 80-180 degrees. (Note that all the angular range is traceable with scanning flow cytometer; part of the range near 90 degrees is traceable with sideward scattering (SSC) channel of conventional flow cytometer). Within the angles range, the non-smooth cells scatter light stronger then smooth ones and intensity of scattered light for non-smooth and smooth lymphocytes are roughly differed by no less than 1.1 order of magnitude. This difference is quite enough to be revealed, for instance, by light scattering channels of flow cytometer, in particular, by SSC channel of conventional flow cytometer.

Aiming at the simple cell model, we neglect by some structure features of the cells. In particular, we replace a slightly rough cell surface by ideal spherical one. To determine the effect of such a replacement on particle light scattering we use the known literature data and demonstrate that local roughness of the cell surface generating scatter in the fashion of very small Rayleigh scatterers has not a dominant impact on scattered intensity. For the nonsmooth cells dominant impact for increased scattering is due to the thickness of equivalent coated sphere.

The lymphocyte model developed in this paper takes no notice of cell nucleus since the results of our angular pattern calculations demonstrate that it is acceptably to ignore this organelle in the context of the problem involved. Having analyzed the known data on the relative importance of mitochondria and lysosomes in lymphocyte light scattering, we conclude that these cell organelles can be ignored in the frame the problem involved as well.

As mentioned above, intensity differerences in light scattering patterns calculated in the frame of the constructed model are sufficient to see contrast between the lymphocytes of healthy and infected individuals in the scattering-angles range of 80-180 degrees. Light scattered near the scattering angle 90 degrees can be measured by the side-scattering (SSC) channel of conventional flow cytometer. We compare SSC signal distributions measured with conventional flow cytometer for cells of lymphocytes populations of healthy and infected individuals (Figs. 1, 2). The comparison shows that for infected individuals as compared with healthy ones the distribution is shifted to large SSC intensities. The experimental data support the results of our calculations for smooth and nonsmooth lymphocytes of healthy and infected individuals. It is worth noting that the shift of scattered intensity distribution for infected individuals as compared with healthy ones will be larger when measuring scattered light by scanning flow cytometer rather than by conventional instrument used in our experiment. The fact is that scattering intensity differences between smooth and nonsmooth lymphocytes are larger in the backward direction than in sideward one (recall that measuring of scattering in backward direction is accessible for scanning instrument and inaccessible for conventional one). For example, the difference near the scattering angle 165 degrees is about 4.5 times larger than the difference near the scattering angle 90 degrees as demonstrate the results of our calculations of scattering patterns for nonsmooth and smooth lymphocytes. Experimental results show that the indicated changes of the SSC signal distributions can be used as additional parameter to be alert to a disease under primary detection of individuals infected with viruses of hepatitises B and C. The proposed approach could be utilized to screen individuals of risk groups, by conventional or wide-angle flow cytometer under the real-time mode, for detecting the persons suspected as infected ones with some viruses.

Thus, calculations have demonstrated that intensity differences of sideward and backward scattered light enables one to distinguish the smooth and non-smooth lymphocytes. The cells can be distinguished by the scattering patterns with the scanning or conventional flow-cytometers. Measuring of backward scattered light with scanning flow cytometry gives more opportunities for distinguishing the cells as compared with the conventional flow-cytometry as scattering intensity differences between smooth and nonsmooth lymphocytes in the backward direction are larger than in the sideward one (for example, the difference at the scattering angle 165 degrees is about 4.5 times larger than the difference at the scattering angle 90 degrees).

The results of the calculations are confirmed by the experiment with a conventional flow cytometer for lymphocyte populations of infected and healthy individuals. It is experimentally shown that the sideward-scattering intensity distribution for lymphocytes is a preliminary signature of some virus infections. The experimental results support our model of lymphocytes for angular patterns of healthy and infected individuals. The proposed approach can be utilized to screen individuals of risk groups by immunophenotyping-free conventional or scanning flow cytometry for inexpensive and rapid provisional detecting of persons suspected as the persistently infected with viruses of hepatitises B and C.

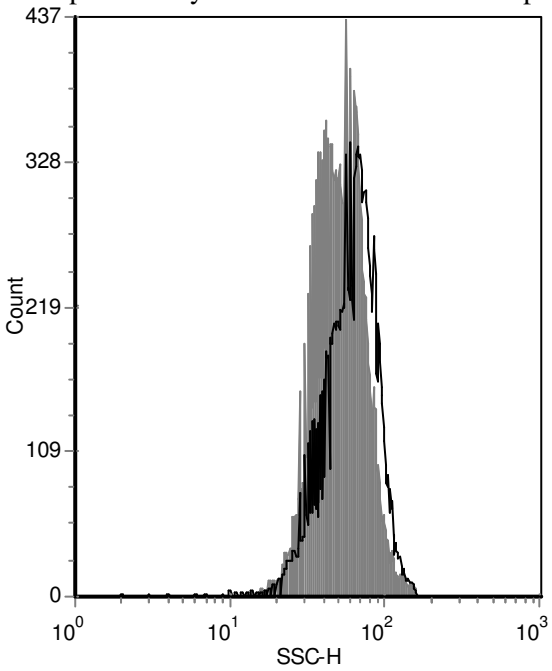

Figure 1 – Normalized histograms of side-scattering signal distributions measured with FACScan flow cytometer and analyzed using the CellQuest software (Becton Dickinson) for lymphocytes of healthy individual (gray) and patient with persistent hepatitis C (black).

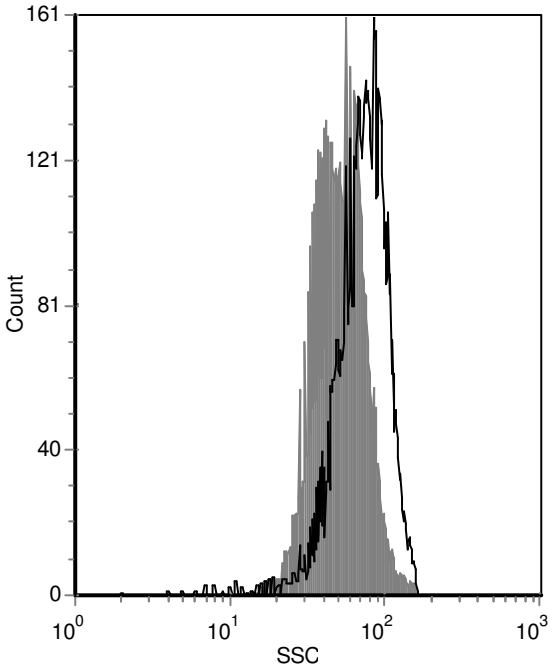

Figure 2 – Normalized histograms of side-scattering signal distributions measured with FACScan flow cytometer and analyzed using the CellQuest software (Becton Dickinson) for lymphocytes of healthy individual (gray) and patient with virus hepatitis B and hepatic cirrhosis (black).

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