# Mononuclear cells morphology for cells discrimination by the angular structure of scattered light

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**Abstract**

The morphology of peripheral blood mononuclear cells (lymphocytes and monocytes) of normal adult individuals is investigated by the methods of specialized light microscopy. The geometrical parameters of cells are analyzed. The possibility of optimization of mononuclear cells separation by light scattering is discussed. Obtained results can be used for the cells discrimination by the angular structure of scattered light.

## 1 Introduction

Biological particles can be characterized by the angular structure of scattered light. Flow cytometry is a modern technique for particle identification, which deals with the light scattering data. It is widely used for diagnostics of different diseases as well. In flow cytometer the particles are analyzed with the rate up to 5000 per second. In conventional flow cytometers the cells are identified by the intensity of forward ($1°<\theta<3°$) and sideward ($65°<\theta<115°$) scattered light and fluorochrom emission. In the scanning flow cytometer the cells are classified by the intensity of light scattered in a wide interval of angles ($5°<\theta<120°$) [1, 2].

The angular structure of scattered light strongly depends on geometrical and optical particle parameters, namely, size, shape, internal structure, and refractive index. The aim of our investigations is to give a detail description of cells morphology to solve the problem of cells discrimination by the angular structure of scattered light [3]. In our previous work the morphology of lymphocytes was investigated [4]. Here the data on monocytes are presented and the comparison of geometrical parameters of lymphocytes and monocytes is carried out. The possibility of mononuclear cells discrimination by light-scattering profiles is discussed.

## 2 Cells morphology. Results and discussion

Monocytes of peripheral blood of healthy individuals are investigated by the methods of light microscopy using a Leica DMLB2 microscope. A cellular suspension is sandwiched between the object plate and cover slip as in a microcuvette. To recognise monocytes CD14 –phycoerythrin –staining is used. The differential interference contrast and fluorescence modes are applied. The lens with $100^X$ magnification and numerical aperture of 1.25 are used. We analyse cell morphology with a Leica image processing software IM 1000. The image of peripheral blood monocytes is presented in Figure 1.

Our observations show, that the shape of lymphocyte and lymphocyte nucleus is slightly elongated or round. Usually the nucleus occupies the most part of lymphocyte (about 80%). The mean value of the ratio between the major axes (maximum linear sizes) of lymphocyte and its nucleus is 1.2. Lymphocyte sizes vary in the range from 4.8 to 11.8 microns. The lymphocyte nucleus sizes vary in the range from 4.1 to 8.3 microns. The mean value of lymphocyte size is about 7.5 microns and the mean value of lymphocyte nucleus size is about 6.2 microns [4].

The shape of monocyte is usually round, but the shape of monocyte nucleus is more complicate. The nucleus occupies about 65% of monocyte. The mean value of the ratio between the major axes of monocyte and its nucleus is 1.5. The sizes of monocytes and their nuclei are measured. The histograms of size distribution of monocytes and their nuclei are constructed. The mean value of monocyte size is 9.87 microns and the mean value of monocyte nucleus size is about 6.44 microns. The investigation demonstrates that the monocyte nucleus is eccentric as well as the lymphocyte one. The intervals of lymphocyte, monocyte, and their nuclei sizes are overlapped.

Figure 1: Peripheral blood monocytes.

Peripheral blood mononuclear cells can be identified by the angular structure of light scattered in the forward hemisphere. The difference in morphology of lymphocytes and monocytes results in the sideward scattering. To separate lymphocytes and monocytes the intensity of sideward scattered light is used. To

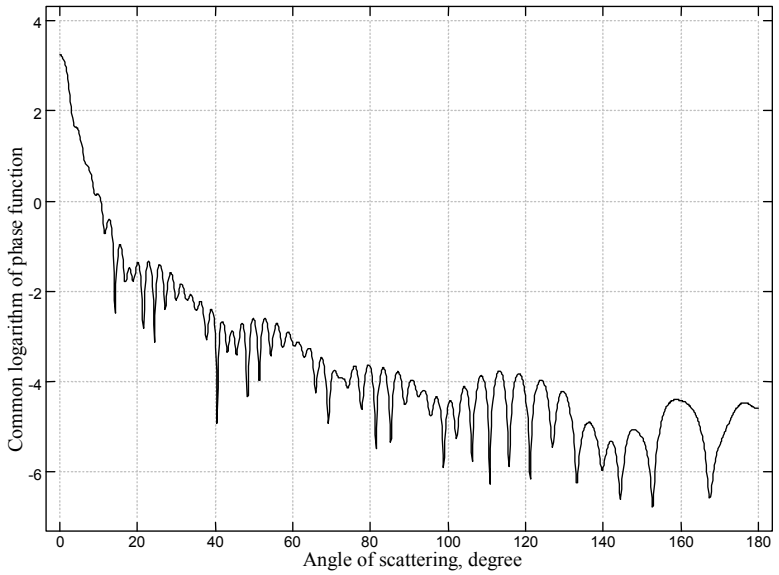


Figure 2: The calculated angular dependence of light scattering intensity for lymphocyte. Diameter of cell is 10.4$\mu$m, diameter of nucleus is 8.2$\mu$m, refractive index of cell is 1.37, and refractive index of nucleus is 1.39. Refractive index of medium is 1.35. Wavelength of incident light is 0.633$\mu$m.

optimize the problem of the cells identification, it is important to estimate the range of angles where the difference in light scattering patterns of lymphocytes and monocytes has maximum. We simulate the scattering from mononuclear cells by bi-layered spherical particles [5] (cell with nucleus) using the results of our experimental data on sizing of the cells. Obtained results show that, the main difference in scattering profiles of lymphocytes and monocytes takes place in the backward hemisphere. It is determined by the difference in cell-nucleus ratios of the cells. As we indicated above, the mean cell-nucleus ratios for lymphocyte and monocyte are 1.2 and 1.5, respectively.

The angular dependences of light scattered by bi-layered spherical particles with close external diameters are shown in Figures 2 and 3. These figures demonstrate that the cell-nucleus ratio has the maximal effect on the light scattering intensity in the range of angles from 125 to 150 degrees.

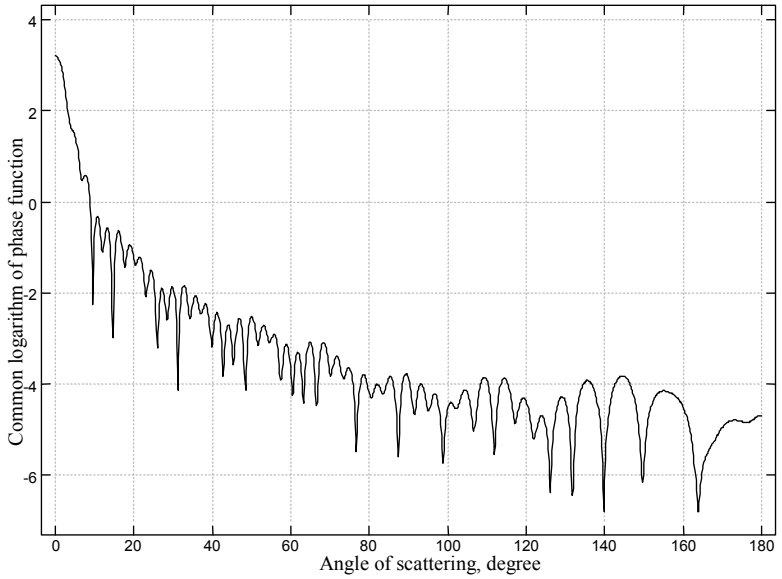


Figure 3: The calculated angular dependence of light scattering intensity for monocyte. Diameter of cell is 9.87$\mu$m, diameter of nucleus is 6.44$\mu$m, refractive index of cell is 1.37, and refractive index of nucleus is 1.39. Refractive index of medium is 1.35. Wavelength of incident light is 0.633$\mu$m.

## 3 Conclusion

To solve the inverse light scattering problem [6-8] of cells discrimination the optical models of cells are necessary. For construction the adequate optical model one has to know the cells morphology. The peripheral blood mononuclear cells of healthy adult individuals are investigated by the methods of specialized light microscopy. The geometrical parameters of lymphocytes and monocytes are presented. It is shown, that the cell-nucleus ratios for lymphocyte and monocyte are noticeably different. It results in the angular patterns of scattered light mainly in the backward hemisphere. The obtained data can be used to optimize the mononuclear cells discrimination by the light scattering intensity.

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