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# A new optical model of mononuclear cells for detail characterisation of morphological changes during early stages of apoptosis

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Apoptosis is the process of programmed cell death implicated in biological processes ranging from embryogenesis to ageing, from normal tissue homoeostasis to many human diseases. Therefore researches of this process are very important for medicine and open new perspectives in the cell biology and immunology. Usually apoptosis are identified by biochemical or immunological methods. However such methods may have an undesirable effect on the object studied. On the other hand, Apoptosis is characterized by significant morphological changes of cellular nucleus which can be measured with non-invasive optical methods. Therefore this work is devoted to present a new fluorescence-free technique for the kinetic study of the early stages of apoptosis by means of measuring morphological changes of mononuclear cells population with a scanning flow cytometer and a new optical model of mononuclear cells.

Experimental basis of this work is scanning flow cytometry technique that allows one to study the light scattering of individual cells. Solving the inverse light scattering problem one can obtain some characteristics of cell. In our previous work we applied a bilayer sphere as an optical model of a mononuclear cell. In this work we used a bilayer sphere with the eccentric inner sphere that allows us to take into account the nucleus heterogeneity. In this study we used lymphocyte samples obtained with a density-gradient separation procedure from the whole human blood. Lymphocytes were analyzed before induction of apoptosis and during 3 hours after induction.

The dynamics of nuclear cells volume distribution functions has been received during early stages of apoptosis. Applying the function proposed by us for the processing of the experimental kinetic data we calculated such parameters of apoptosis as the fraction of apoptotic cells, the characteristic time of the apoptosis lag-phase, and the cell population synchronicity to go into apoptosis.

Thus we presented a new fluorescence-free method for the kinetic study of the early stages of apoptosis. This method allows one to determine next mononuclear cells characteristics: the volume of cells and cell nuclei before and after the initiation of apoptosis, refractive index of the nucleus, the fraction of apoptotic cells, the characteristic time of the apoptosis lag-phase and the cell population synchronicity to go into apoptosis. The use of a new optical model enables us to take into account the lymphocyte nucleus heterogeneity and the chromatin condensation at the nuclear periphery during apoptosis by that increase the accuracy of determining cells characteristics.