

COHERENT PHASE MICROSCOPY ENABLED DETERMINATION OF A SINGLE T-LYMPHOCYTE'S FUNCTIONAL STATE

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Refractivity of cellular organelles can be used as an indicative parameter to show cell functional state and an individual's response to drugs and energy depletion. The new rapid method of *in vitro* immune monitoring based on measurements of Refractivity of Cellular Organelles (RCO) is proposed. Here we discuss the results of T-lymphocyte refractivity measurements using the Coherent Phase Microscope "Airyscan". The phase images of cells provided additional information about their morphology. Methods of laser-based interference microscopy combined with some assumptions enable measurement of refractive indices (or refractivity) of biological objects which are dependent on their functional state. In this work, T-lymphocyte phase images were registered by means of coherent phase microscopy method. Phase thickness values of the lymphocyte in characteristic points of its phase image histogram and its optic model were used to calculate the refractivity of the T-lymphocyte's organelles. In this simulation, the T-lymphocyte was presented as a semi-ellipsoid, and its organelles as layers of different thickness and refractivity. The phase thickness $h(j,k) = \sum H_i(j,k) \Delta n_{0i}$ in characteristic points (j,k) of the histogram was presented as a sum of i-x layers (i = 1,2,3,4) of the known geometric thickness $H_i(j,k)$. Here n_0 is the refractive index of the outer (immersion) medium, and the organelles' refractivity $\Delta n_{0i} = n_i - n_0$ are denoted by the indices: 1 - for the peripheral cytoplasm, 2 - for the denser part of the cytoplasm near the nucleus, 3 - the nucleus, 4 - the nucleoli. The solution of the system of equations for the known geometric thickness of the layers allowed us to obtain refractivity values $0,005 < \Delta n_{01} < 0,01$; $\Delta n_{02} = 0,075-0,08$; $\Delta n_{03} = 0,08-0,09$ и $\Delta n_{04} = 0,09-0,11$. The biophysical significance of organelle refractivity as functional state parameters was confirmed in the following experiments. Rotenone addition into the medium to block the first complex of mitochondrion respiratory chain resulted in the abrupt reduction of refractivity (Δn_{02}) of the dense part of cytoplasm in 30 minutes. Activation of T-lymphocytes from healthy donors by laser radiation was accompanied by the reduction of refractivity of the chondrion and the nuclei. Prednisolone blocked the activation that usually took 10 min. in T-lymphocytes from healthy donors under laser radiation. The radiation did not affect the lymphocytes of sick donors resistant to prednisolone. Our research allows us to conclude that organelle refractivity provides useful information and, in principle, enables rapid diagnostics of an individual's immune response on the cellular level.

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