

IDENTIFICATION OF BLOOD CELL SUBPOPULATIONS FROM ANGLE RESOLVED LIGHT SCATTERING

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Flow cytometry has experienced a considerable expansion of available parameters during the last years. Various techniques that allow for the direct analysis of leukocytes by direct labeling with antigen-specific fluorescent labeled antibodies or according to functional parameters will have enormous impact on immunological research and hematopathological diagnosis. Scanning flow cytometry (SFC) [1] with its additional ability to measure morphological and/or biophysical differences between different blood cell subpopulations can make analysis of leukocytes more accurate and more comfortable. The angle resolved light scattering pattern (LSP) measured with SFC contains encoded information about morphological and biophysical properties of cells. Cells having subtle differences in their morphological and/or biophysical properties can potentially be better discriminated by LSP compared to “classical” integrated side scatter measurements. Eventually this could lead to a reduction in the number of antibodies needed for proper subset identification, thus allowing for either less expensive testing or adding additional antigenic evaluations to the test.

The aim of this study is to develop multiparametric methods of classification for peripheral blood (PB) cells based on the LSP only and using them for cell subset identification. We adapted Bayes classification algorithm to classify different types of blood cells. The antigen-specific (antibody) labeling was used for building of a learning sample but not for the classification itself. The integrals of LSP over different angular ranges and spectrum parameters of LSP were used in the classification. The optimization of algorithm was done by correctly defining parameters. We verified the obtained algorithm on different healthy donors. Though some overlap in cell subsets is present, good evaluations with subset classification errors less than 15% can be made without the need for antigen-specific labeling.

References

- [1] Maltsev V.P. and Semyanov K.A., Characterization of Bioparticles from Light Scattering (Vista Science Press, Netherlands, 2004).



CHARACTERIZATION OF MONONUCLEAR BLOOD CELLS FROM LIGHT SCATTERING

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Modern diagnostic equipment based on classical flow cytometric techniques developed rapidly into a cell subset classification tool based on the simultaneous evaluation of several (up to 5 and more) cellular antigens detected by the use of fluorescent labeled monoclonal antibodies. The two light scattering parameters (collected in the forward and side direction) are used for the discrimination of the major leukocytic populations (lymphocytes, monocytes and granulocytes).

Scanning flow cytometry (SFC) allows for measuring the light scattering intensity dependency on detection / observation angle, i.e. the light scattering indicatrix (LSI) in range from 10 to 70 degrees. The LSI contains information about morphological and biophysical properties of cells: size, shape, internal structure, sizes and refractive indices of cytoplasm, nuclear, organelles, etc. In this way the LSI can be regarded as comparable to a microscopic image but with encoded information content. The additional information thus obtained can help in the detection of aberrant (pathological) versus normal cell types.

In order to obtain information about the morphology of a cell it is necessary to solve the inverse light scattering (ILS) problem. Most blood cells are far from homogenous and spherical: unfortunately there are no methods available at the moment for solving the ILS problem for inhomogeneous and nonspherical cells. This study is the first step in attempting to solve the ILS problem for blood mononuclear cells.