CYTO 2010

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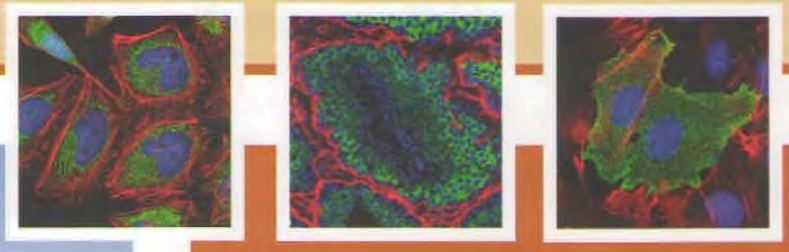

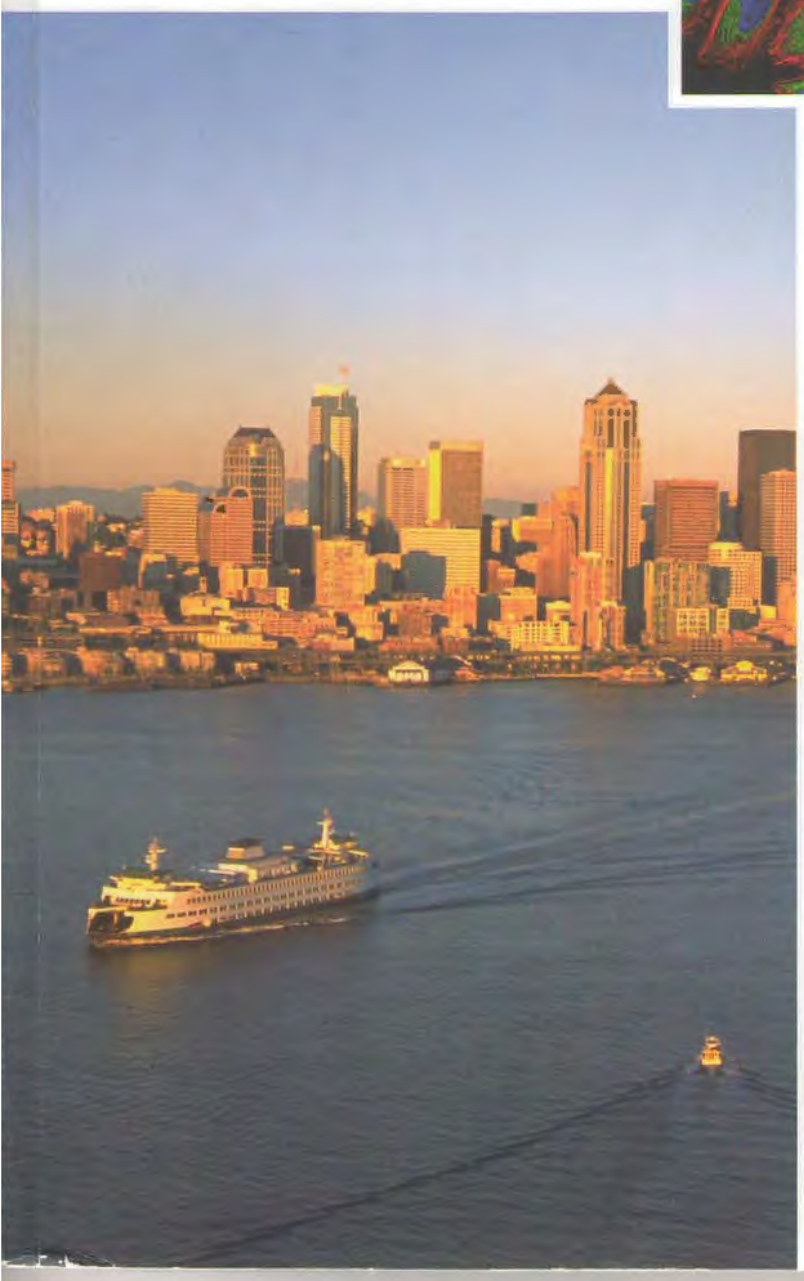

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DEVELOPMENT OF 10-COLOR FLOW CYTOMETRY PROTOCOLS FOR CLINICAL STUDIES

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Background: Nowadays cytometric industry is booming. With synthesis of novel fluorochromes, innovative light sources and optic filters, chemistry and physics create new possibilities and more effective substitutes for old ones. These latest developments lead to a new generation of commercial clinical flow cytometers (FCM) that are equipped with three (blue, red, violet) or more lasers and multitude of PMT detectors. Actually some routine clinical instruments capable of detecting 10 or more fluorescence colors simultaneously have in vitro diagnostic certification and are suitable for a GLP environment. They present opportunities for getting detailed information on single cell level for cytomics and systems biology based improved diagnostics and monitoring of patients.

The University of Leipzig (Germany) recently started a cluster of excellence to study the molecular background of life style and environment associated diseases enrolling 25,000 individuals. For this study a most comprehensive FCM protocol of leukocyte immunphenotyping has to be developed that allows large scale immunophenotyping. 10-color FCM enables for increased accuracy in cell subpopulation identification, the ability to obtain detailed information from blood specimens, improved laboratory efficiency, and the means to consistently detect major and rare cell populations **Methods:** We performed detailed analysis of fluorochromes and antibodies from different providers in order to compare and optimize reagent combinations to the characteristics of the instrument for successful 10-color FCM in this clinical setting. Systematic review of issues related to sampling, preparation, instrument settings, spillover and compensation matrix, specific and unspecific labeling and other reagent performance, and general principles of panel construction was performed. **Results:** We developed 10 color staining protocol for leukocyte immunophenotyping showing major and rare cell subpopulations with quantitative characterization of their abundance and activation. **Conclusion:** Careful attention to details of instrument and reagent performance allows for the development of panels suitable for screening of samples from healthy and ill donors. The characteristics of this technique are particularly well suited to the analysis in broad human population cohorts and have the potential to reach the everyday practice on standardized way in the clinical laboratory.

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SCANNING FLOW CYTOMETRY IN MEASUREMENT OF LYMPHOCYTE MORPHOLOGY: CELL AND NUCLEUS SIZES, CYTOPLASM AND NUCLEUS REFRACTIVE INDICES

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Background: Modern flow cytometers are focused mainly on cell identification and have a weak performance in cell characterization, i.e. in determination of physical characteristics of cells. In particular, blood platelet volume and refractive index, red blood cell volume and hemoglobin concentration are the only physical characteristics that can be measured reliably with commercial cytometers. Nevertheless, morphological analysis and automatic cell sizing remain one of the key points in studying, diagnosing and classifying lymphoproliferative disorders. Over the last decade scanning flow cytometer (SFC) has been developed, which is capable to acquire more physical characteristics of individual particles, by measuring detailed light-scattering profiles and inverting them relying on dedicated optical models. **Methods:** Preparation of lymphocyte samples was carried out by a density-gradient separation procedure. Labeling with immunofluorescence markers CD3-FITC and CD19-PE were used to identify T- and B-lymphocytes respectively. LSPs of individual lymphocytes were measured with the SFC. We solved the inverse light-scattering problem for lymphocytes, using a model of a coated sphere and optimization algorithm. Light scattering by a coated sphere model can be quickly computed using the Mie theory. To perform global optimization of model parameters we use the DIRECT algorithm and propose a method to compute errors of the parameter estimates. **Results:** We characterized T- and B-lymphocytes from several donors, determining cell diameter, ratio of nucleus to cell diameter, and refractive index of the nucleus and cytoplasm for each individual cell and determined distributions over cell characteristics. We obtained the lymphocyte diameter from 5.7 μm to 7.4 μm, the refractive index of the cytoplasm from 1.37 to 1.38, the refractive index of the nucleus from 1.43 to 1.46, and the means cell sizes of T- and B-cells are 6.3 μm and 6.6 μm, respectively. **Conclusions:** We developed a method to characterize lymphocytes from light-scattering patterns measured with the SFC. This method can be easily adapted to any mononuclear cells. Our results for cell and nucleus diameter fall within the broad range of literature data and there is an unusually small variation in mean size of T-cells and their nucleus from donor to donor. The main difference in morphology of T- and B-lymphocytes was found to be the larger mean diameters of the latter. Although the difference in mean cell size of about 5% is statistically significant, it is smaller than the estimated biological variability 0.6 μm inside each sample

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INVITRO PRODUCTION OF ANTI LPS ANTIBODY BY PERITONEAL AND SPLEEN B-LYMPHOCYTE OF BALB/C MICE

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Background and Aim: B1 lymphocyte subpopulation is the most dominant lymphocytes in defence surface of many organs: peritoneum and splenic follicular lymphoid compartment. At the time of need they can produce natural antibodies with poly specific reaction with one of the important Ligand: Lipopolysaccaride (LPS). The Aim of this study was to be possible of isolation and purification of this exclusive and unique population from cellular content of peritoneum/ spleen and blood and comparison of functional activity of them to produce IgM against stimulant under cell culture and experimental condition. **Material/Methods:** With direct heart puncture and harvest cells with peritoneal lavage from inbred Balb/C mice to take action for harvest B cells with Ficole density gradian and nylon wool column for purification of B lymphocytes. In complete tissue culture medium, cells divided into 2 Groups: case and control with LPS stimulation in different time of duration: 24/48/72 hours incubation, Finally culture suppernats were assess for IgM concentration with ELISA Technique. The prolifenation rate was defined by M.T.T assessment. Immunophenotyping studies for confirmation of cellular purity were carry out by CD3 and CD5 markers. **Result:** Lymphocytes from spleen and peritoneum organs had highest level of IgM secretory activity in 24 hourse, with significant differences versus control groups. In Immunophenotypeing study, purified Blymphocytes from Peritoneum showed Highest level of CD5 marker. **Conclusion:** The findings of this research indicate that the production of IgM with polyspecific properties against LPS in laboratory condition have been nicely through in a good way. Peritoneum is a very pure and available source of B-1 lymphocyte to take easy for isolation. The defect of blood circulation from these cells and low power of IgM secretion revealed that spleen and peritoneum have a high