pump laser and gas configurations were assembled as excitation sources on a BD LSR II and modified FACSort at both the NIH in the USA and the XLIM Centre in France, and used to excite a variety of fluorescent probes, including PE and APC on MESF microspheres, and cell lines expression several red fluorescent proteins including DsRed and tdTomato. In all cases, the laser lines produced from HC-PCF sources gave equivalent excitation to conventional single wavelength lasers.

While still an early technology, HC-PCFs have the potential to produce many useful, discrete laser lines from a single laser source, making them another step in the "any excitation wavelength, any emission wavelength, any probe" paradigm of flow cytometer design.

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Determining Intracellular Protein Localization with Fluorescence Lifetime-Based Flow Cytometry

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Background: An important role in the normal biological activity of proteins is theirlocalizationto different regions inside the cell. Protein localization can be readily measured with microscopy using standard fluorescence or immunofluorescence-based techniques. Flow cytometry can also detect protein presence, howeversubcellular changes in protein location are difficult to measurewithoutimage-based approaches. In this contribution, we introduce fluorescence lifetime-based cytometry as a way of indicating protein movement inside the cell at a high-throughput level. That is, changes in the fluorescence lifetime of fluorescent proteins are measured when bound to other proteins of interest, whose subcellular locationsare altered. By detecting the fluorescence lifetime changes, we are able to indirectly measure two key protein localization and mislocalization events: (1) the nuclear to cytosoliclocalization of the p27 cell cycle regulator, an event which is correlated with cell cycle progression and also, carcinogenesis; and (2) the localization of the LC3 protein to particular regions of the cytosol to form autophagosomes, a subcellular characteristic of autophagy.

Methods: Enhanced green fluorescent protein (EGFP) was chosen for all protein localization studies. EGFP was expressed as a tagged partner to different proteins using a breast cancer cell line(MCF-7). The tagged constructs includedEGFP-LC3, EGFP-mLC3 (G120A, a mutant protein that is unable to localize at autophagosome) and EGFP-p27.To induce subcellular localization of the LC3 and p27 proteins, amino-acid and serum deprivation was applied. Also, nucleo-cytoplasmic, or otherdistribution of the LC3 and p27 proteins was confirmed through confocal microscopy and cellular fractionation. The average fluorescence intensity and fluorescence lifetime were measured with an Accuri flow cytometer as well as a home-built fluorescence-lifetime based flow cytometer, respectively. Finally, protein levels were measured by western blotting.

Results: The fluorescence lifetime value of EGFP was changed when localized into different subcellular regions of the MCF-7 cells. The fluorescence lifetimes changed to a larger extentthan the average fluorescence intensity because the average concentration of the EGFP remained the same despite its location within the cell.When the EGFP-LC3 protein localizedinto autophagosomesand formed punctate regions during amino-acid starvation, the average fluorescence lifetime became shorter compared to the protein in a diffuse cytoplasmic state. We observed many fluorescence lifetime changes (3-ns to 10-ns) for several different controls ranging from mutant LC3, non-starved cells, cells with only EGFP, cells with no EGFP, etc. Additionally, when the p27 protein localization was studied a range of 3 to 4 ns fluorescence lifetime changes were observed.

Conclusion: The fluorescence lifetime is a quite powerful photophysical trait that indicates differen tchemical and biochemical environmentsof fluorophores. In the context of subcellular protein localization, we envision a powerful high-throughput technique for drug and target screening. Future work will be to evaluate the lifetime sensitivity and resolution and introduce other protein localization events. This study was supported by a New Mexico State University Interdisciplinary Research Grant by the Office of the Vice President for Research.

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A Label-Free Shape-Based Detection of Activated Platelets with Scanning Flow Cytometry

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Background: Blood platelets are involved in many diseases, including myocardial infarction, stroke, peripheral vascular disease, cancer, and many infections. Their hyperfunction, especially inappropriate platelet activation, plays a prime role in the increasing heart-disease burden of society. It is, therefore, important to assess platelet activation. There are several approaches to detect activated platelets measuring the expression of surface receptors with the flow cytometry. We propose an alternative approach based on the measurement of platelet shape, also being a marker of platelet activation. This measurement is possible with the scanning flow cytometry and does not require labeling. Hence, it is promising for clinical analyses due to performing the test instantly after the venipuncture.

Methods: We used the scanning flow cytometry, which is based on the measurement of angle-resolved light-scattering patterns (LSPs) of individual cells and on the solution of the inverse light-scattering (ILS) problem. We measured LSPs with the Scanning Flow Cytometer fabricated by CytoNova Ltd. (Novosibirsk, Russia, http://cyto.kinetics.nsc.ru/).The solution ofILS problem is based on fitting an experimental LSP with theoretical ones, modelingplatelets as oblate spheroids.Thus volume and shape of individual plateletsin a sample are determined.

In addition to measuring platelet LSPs, we also performed antibody (anti-P-selectin) labeling to correlate shape and surface antigen expression of platelets. We used several agonists (ADP, collagen, and thrombin) to induce platelet activation. Flow-cytometric measurements were accompanied by microscopic observations and Coulter analysesas independent controls.

Results: The study of platelets with the scanning flow cytometry wasperformed for several donors. The platelets volume and shape determined by the solution of the ILS problem were in an agreement with the corresponding data from microscopic observation and Coulter analyses. The determined platelet shape showed a correlation with the P-selectin expression after stimulation by several agonists.

Conclusions: The shape-based approach to detect activated platelets is presented. It does not require labeling step, which facilitates rapid tests in clinical setting. High precision in measurement of platelet axis ratio and volume opens a way to study the kinetic details of platelet activation. The results of the comparison with existing methods are presented.