Time resolved measurements of the conformation changes of cellular structures using fluorescence polarization flow cytometry

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The polarization of the emitted light is a measure of the rotational mobility of the fluorescent molecule if energy transfer can be neglected. Changes of the conformation of intracellular structures or of their fluidity can be monitored using fluorescent tags. The fluidity of membranes and cytoplasmic structures of intact cells have been investigated by either single cell or bulk analysis using a cell suspension. A single cell technique allows the evaluation of the polarization independent of the fluorescence intensity. For sufficient data collection flow cytometric techniques provide the necessary tools. However conventional flow cytometers give raise to a number of systematical artefacts. We therefore introduced an epi-illumination flow system that avoids most of the problems of proper imaging (1). We have expanded the possibilities of our system by allowing each cell to flow through two laser beams. By using perpendicular polarization of the exciting beams the degree of polarization of each cell is measured twice within 40 µsec with the same set of detectors allowing an internal correction of system asymmetries. A dedicated microprocessor system is employed for the internal correction as well as for fast calculation of the degree of polarization of the individual cell. This system allows monitoring of changes of cells within the time scale of a few seconds. We have been able to follow the melting characteristics of lipid vesicles (50-100 Å) during heating on a one by one basis as well as the alteration of cellular structures after stimulation. Since conventional parameters such as cell diameter, total fluorescence intensity or surface structure remain available, fluorescence polarization analysis can be used for a variety of cell biological stimulation experiments.

1) Eisert, W.G. and Beisker, W.; Biophys. J. 31, 97-112 (1980)