Fluorescence Polarization measurements on synchronized Hela-cells and human lymphocytes. A single cell study using flow cytometry.

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Fluorescein which is produced from the fluorogenic substrate fluorescein-diacetate (FDA) in the cell measures unspecifically the fluidity inside the cell. This was proposed by Cercek et al.[1] for the investigation of lymphocyte stimulation and cell cycle kinetics in cell suspensions and was adapted by our group to flow cytometry avoiding disadvantages like background fluorescence, multiple scattering and averaging over subpopulations. For this purpose a flow cytometer with some special properties was developped:

1) The flow chamber with an asymmetrical alignment of the central steam allows the use of oil objectives with high numerical aperture for the measurement of weak fluorescences. The chamber fits as well in every microscope fluorimeter. The cells are illuminated in an epi-arrangement by an Ar-ion laser, respectively an arc mercury illuminator.

2) Common microcomputers like the Apple II are well suited for the data management of flow cytometers. With a plug-in A-D interface measurement rates with one or two parameters up to 10 kHz are possible. The microcomputer plots two- and three dimensional histogramms and computes mean polarization and intensity values.

By using more polar carboxyfluorescein which is retained better in the cells fluorescence polarization spectra could be measured continously. Maximal changes of the degree of fluorescence polarization after lymphocyte stimulation could be found at λ_{ex} = 476 nm and λ_{em} = 510 nm.

Cells from Hela-cultures were synchronized by mitotic selection. The degree of polarization (P) is dependent of the FDA concentration and the incubation time. High FDA-concentration (10^{-6}m) results in high fluorescence intensity which averages out all differences between the cells. At 10^{-7}m FDA P is lowered from 0.16 to 0.11 during 8 hours after mitotic selection. The mean fluorescence intensity is highered in this time by a factor of two.

Lymphocytes after stimulation with PHA in the early G1-phase show a similar reduction in P. Measurements on the lymphocytes of 50 healthy donors give reductions in P from 10 - 30 % after 1 hour PHA stimulation. There is evidence that in some cases of malignant deseases the stimulation by PHA has no effect on the degree of polarization.

1) Cercek et al. Europ. J. Cancer, 13, 903-915 (1977)
2) W. Hartmann et al. Biomedicine, 32, 185-188 (1980)