Chapter 1

Operation of a Flow Cytometer

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Introduction

Biomedical research, from the organism to the molecule and back again, requires powerful tools to analyse the functional status of individual cells, the unit of organisation of life. Cells have been detected by microscopy which, in combination with powerful staining technologies, is still the main instrument to obtain direct information on their state of activation, proliferation and differentiation. The drawback of microscopy is that the data generated are mainly “visual impressions“ and not exact numbers. Today, systems are available that allow quantification of light intensity of microscopic objects, but these systems are still too slow to allow analysis of enough objects to obtain good statistics. One could say that microscopy generates too much information in cases, where only information on the amount of a particular stain per cell is desired.

The idea of measuring high numbers of stained cells quickly and precisely was realized between 1965 and 1970 by combining electronic light measurement and the concept of letting a flow of small cells pass the microscope’s objective rather than moving the large microscope over the cells [1,2]. This basic concept of flow cytometry is realized in a variety of commercially available instruments, like Aber Instruments MICROCYTE, Becton Dickinson’s FACScan, FACStrak, FACSort, FACSCalibur, FACStar, FACSVantage, Bio-Rad’s BRYTE-HS, Coulter’s PROFILE and EPICS, Cytomation’s MoFlow, Ortho’s CYTORON and last, but not least, Partec’s PAS.
machines. For the hobby-engineer, Shapiro has described a build-it-yourself cytometer, the CYTOPUP and CYTOMUTT [2].

The application range of flow cytometry has grown ever since its original introduction, mainly due to the development of powerful staining technologies that make use of the high sensitivity of fluorescence, the principle of chromogenicity, i.e. color shifts caused by biochemical reactions of the dye, and monoclonal antibodies to stain cells for specific proteins. Methods are available to quantify DNA content very precisely (→ Kap. 10), determine the proliferative history of a cell in vivo (→ Kap. 11) and in vitro (→ Kap. 12), to measure ion fluxes correlated to the cell’s physiology (→ Kap. 13, → Kap. 14, → Kap. 15) and enzymatic activities, and to discriminate cells in complex mixtures by immunofluorescence (→ Kap. 2, → Kap. 3, → Kap. 4).

Here, we describe the principal components of flow cytometers, the fluid system that transports the cells across the microscopic field, the optics of illumination and detection, and the electronics for light collection, data management and control. Basic setup and calibration routines are given for a typical fixed aligned flow cytometer and a non-fixed aligned flow-in-air cytometer.

Procedures for other machines may vary slightly. In any case, the manufacturer’s instructions should be observed.

Fluidics

The flow system is shown schematically in Figure 1. The stained cells are applied to the cytometer in a “sample tube”. From the sample tube, the cells are transported by air pressure into the flow chamber, which can be a cuvette directly observed by the microscope, or a nozzle which injects the cells into the air and through an area of microscopic observation (flow-in-air systems).

Essential for the success of flow cytometry is hydrodynamic focusing [3] by which the cells are individualized and positioned at the observation point with an accuracy of better than 1 μm. For hydrodynamic focusing, the cell suspension (sample fluid) is injected into a particle free “sheath fluid” in a large diameter tube which flows in a small cuvette or a nozzle. Typical internal cross-section of a flow cuvette is 430 μm x 180 μm (FACScan) and nozzles with orifice diameters from 50 μm up to 400 μm can be selected for flow in air systems.

For most sortings of cells from blood, a 70 μm diameter nozzle is used. The diameter of the sample flow at the injection point inside the flow chamber is reduced from about 200 μm to about 10 μm at the laser intersection