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Flow Cytometry: A High-Resolution Instrument for Everyone

Abstract. *A new flow configuration for flow cytometry has been devised in which a flat, laminar stream of water, containing the stained cells in a narrow central sector, is formed on a microscope cover slip by a pressurized jet of water directed onto the glass at low angle. The stream of cells is viewed by means of a fluorescence microscope with incident illumination and a pulse photometer. Coupled to a multichannel pulse height analyzer, the instrument constitutes a stable and easy-to-operate flow cytometer with a resolution equal to or better than a coefficient of variance of 1.4 percent in measurements of cellular DNA.*

Quantitative measurements of the cellular content of various cell constituents by flow cytometry is becoming widely used for both research and diagnostic purposes (*1*). The principle of the method is to pass a narrow stream of cells, stained with a fluorescent dye which binds quantitatively to the constituent under study, through a beam of exciting light and to measure the intensity of the resulting pulses of fluorescence. The distribution of cells with regard to such a constituent—for example, DNA—can thus be determined with high precision and at a rate of about 10^3 cells per second. Advanced flow cytometers, which measure several cell parameters simultaneously, are now commercially available. However, there is a growing need for a simple and inexpensive instrument for routine tasks.

One approach to the construction of such an instrument is to utilize a fluorescence microscope (*2*), which provides high aperture optics for both the excitation and the fluorescence light. We describe here a new flow configuration which, when implemented on a standard fluorescence microscope with a suitable photometer, constitutes a relatively inexpensive and easy-to-operate flow cytometer with high resolution and stability.

Our idea is to create a laminar flow on a microscope cover slip by means of a jet of water directed onto the glass at low angle (Fig. 1A). In this configuration, the cells are centered in the jet by hydrodynamic focusing (*3*) and are thereby confined to a narrow and stable section of the flow on the cover slip, which is viewed by an inverted fluorescence microscope in incident light—that is, with the excitation light focused through the detection objective.

The nozzle (Specialty Glass Products part 91216) producing the pressurized jet of water, 80 μm in diameter, is similar to that used in flow cytometers where the exciting light intersects the water jet in air (*4*). The water is drained from the cover slip by suction through a hypodermic needle. The microscope (Leitz Diavert) has a ×40/1.30 oil immersion objective. It is fitted with an incident light illuminator (Ploemopak) and a superpressure mercury lamp (Osram HBO 100) with stabilized power supply (*5*). The nozzle is mounted on the object guide of the microscope stage. When viewed through the microscope binocular, the water jet can thus be readily positioned to obtain the configuration shown in Fig. 1B. A photomultiplier tube (PMT) (EMI 9659QB) is mounted on the phototube of the binocular. A variable slit in the phototube limits the area viewed by the PMT. The pulse length of the PMT is about 5 μsec; thus sample flow rates of the order of 10^3 cells per second can be used. The PMT pulses are fed to a multichannel pulse height analyzer.

The pulse height distribution obtained for rat thymocytes, stained for DNA measurement with a combination of ethidium bromide and mithramycin (*6*), exhibits a symmetrical peak with a full width at half maximum corresponding to a coefficient of variance (CV)—that is, relative standard deviation—of 1.4 percent. A typical plot is shown in Fig. 2. For these cells we routinely obtain CV $\leqq$ 1.5 percent, with no need of readjustments between samples. Since the true CV for these cells is not known, it is not clear to what extent the mesured width reflects the resolution of the instrument. Hence, we conclude that its resolution is equal to or better than 1.4 percent.

The performance of our instrument does not seem to be critically dependent on the exact physical characteristics of the flow system, such as the position and angle of the water jet or its driving pressure (*7*). Hence, optimum conditions are easily achieved and stability appears excellent. The instrument's characteristics are described in detail elsewhere (*8*).

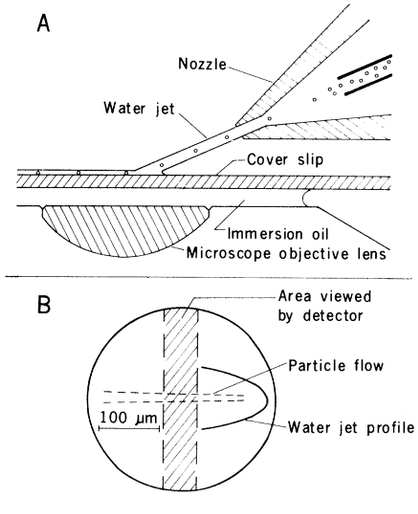

Fig. 1. The essential part of the flow system. (A) A collimated beam of cells in a pressurized water jet, formed by hydrodynamic focusing, hits a glass cover slip at low angle to form a flat, laminar stream with the cells confined to a narrow sector. The cover slip is viewed by means of an inverted fluorescence microscope with incident illumination. (B) The flow as it appears in the microscope. The shaded area indicates the section viewed by the fluorescence detector.

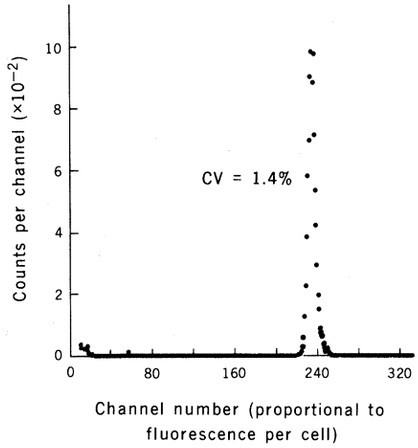

Fig. 2. A plot of the fluorescence (DNA) per cell as obtained for rat thymocytes stained with ethidium bromide and mithramycin, which bind quantitatively to DNA. The full width of the peak at half maximum corresponds to a coefficient of variance (CV) of 1.4 percent. The peak, representing 10,600 cells, was registered in 29 seconds. A small peak, due to cell doublets, was observed at twice the channel number of the present peak.

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The present flow configuration can be used with essentially any fluorescence microscope having incident illumination, whether inverted or upright. Thus, the high speed of the water in the jet (~ 10 m/sec) and the laminar character of the flow on the cover slip allow the system to be oriented in any direction. The great spectral width of the mercury lamp and the large selection of filter combinations available make this type of instrument suitable for a wide variety of stains.

With this flow configuration it is possible for the nonspecialist to assemble, from components that are standard equipment in many laboratories, a flow cytometer that is superior to most with respect to both resolution and simplicity in use and that is capable of making cellular DNA measurements with a CV on par with or better than the best systems commercially available.

Note added in proof: Recently our instrument has consistently yielded DNA histograms with a CV below 1.0 percent (*8*), demonstrating that its resolution is better than this value.

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7. The resolution was independent, that is, CV ≤ 1.5 percent, of the angle α between the water jet and the cover slip in the range α = 15° to 30°; the resolution remained better than CV = 3 percent up to α = 90°, that is, vertical incidence. With α = 20°, the distance between the point where the cells hit the cover slip and the optical axis of the microscope could be varied from 200 to 300 μm without affecting the resolution. The resolution was independent of the driving pressure of the water jet over the range 1 to 4 kg/cm². No special preparation of the cover slip was needed. The limit of resolution appeared to be determined primarily by the stability of the light source. With the 25-V d-c power supply stabilized to within ± 1 mV, the peak channel number of a reference sample remained constant within ± 1.5 percent for several hours. The pulse amplifier was operated with a time constant of 2 μsec. Its linearity, as judged from the position of the particle doublet peak, was within 1 percent.
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Strontium-Calcium Thermometry in Coral Skeletons

Abstract. *The strontium to calcium ratio of skeletal aragonite in three genera of reef-building corals varies as a simple function of temperature and the strontium to calcium ratio of the incubation water. The strontium/calcium distribution coefficients of coral aragonite apparently differ from the corresponding coefficient of inorganically precipitated aragonite. With some care, coral skeletons can be used as recording thermometers.*

Discordant interpretations have been offered to explain the ratio of Sr to Ca in coral skeletons and the variation of that ratio. The extremes of those interpretations may be summarized as follows. (i) Corals precipitate Sr and Ca in the same ratio as these elements occur in seawater (*1*, *2*). (ii) Corals precipitate Sr and Ca in a ratio which varies with taxonomy, metabolism, growth rate, or other biological parameters (*3*, *4*). (iii) Corals precipitate Sr and Ca in a ratio which reflects the seawater ratio modified by a temperature-sensitive Sr/Ca "distribution coefficient," which describes the proportional discrimination of the precipitate for Sr coprecipitation in the aragonite ($CaCO_3$) crystal lattice (*5*).

We carried out controlled laboratory experiments to test the field observations which led to the interpretations summarized above (*6*). Our preliminary findings demonstrated that there is a linear relationship between the Sr/Ca ratio in skeletons of reef-building corals and the ambient water temperature at which the precipitation of skeletal aragonite occurred; that the relationship is not directly growth-rate dependent, except insofar as temperature and growth rate are related; and that there are apparent differences in the Sr/Ca ratio among coral taxa.

We have since examined field data in order to validate our laboratory findings, and we report here field and further laboratory results. These results are easily interpreted in terms of a Sr/Ca distribution coefficient which apparently differs from that of aragonite precipitated inorganically from seawater. Moreover, we have revised the absolute values of our initial Sr/Ca ratios on the basis of what we believe to be an accurately determined Sr/Ca ratio for an internal (coral) standard. Our results suggest that the discordant interpretations of the observed Sr/Ca variation are largely attributable to analytical limitations.

Skeletons of the finely branching coral *Pocillopora damicornis* were obtained from the Pacific coast of Panama (*7*). These corals had been stained in their ambient environment with alizarin red S and then left there to grow at temperatures continuously monitored with recording thermographs. Growth after staining was visible as white coral tips above the pink-stained portion of the skeletons. We analyzed the Sr/Ca ratio of the unstained tips of these corals by atomic absorption spectrophotometry (*6*, *8*). The coral Sr/Ca ratios obtained by this procedure were adjusted by comparison with a coral aragonite standard for which a Sr/Ca value has been established by mass spectrometry. Samples of that standard were routinely analyzed with each batch of coral skeletal Sr/Ca samples.

Because between-tip Sr/Ca variations within a single colony of the Panama corals proved not to differ significantly from variations between repeated analyses of single tips, we pooled tips within subsequently analyzed coral colonies with confidence that we were not losing information about intracolony variability. We then assessed other levels of variability in the coral Sr/Ca ratio. Table 1 presents an analysis of variance designed to examine sources of variability among coral collection sites (*9*) (that is, large-scale environmental variation), between paired coral heads from the same site (small-scale environmental variability or biological differences among corals, or both), and among triplicate analyses of ground-up skeleton from individual coral heads (analytical precision). The analysis demonstrates that 81 percent of the total variability (the sum of the squares among collections divided by the total sum of the squares) represents large-scale environmental variation; 16 per-

Table 1. Analysis of variance of *Pocillopora* Sr/Ca ratios ($\times 10^3$) among collections, between corals at a collection site, and among replicate analyses within corals.

Degrees of freedom	Sum of squares	Mean square	F
	Among collections		
6	1.4172	0.2362	40.7 (significant at $P < .001$)
	Between corals		
7	0.0406	0.0058	0.57 (not significant)
	Within corals		
28	0.2840	0.0101	
	Total		
41	1.7418		

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