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Simple Delay Monitor for Droplet Sorters

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We have constructed a simple device by which the optimal delay time between optical measurement of a cell and the application of the droplet charging pulse can be determined directly in a flow sorter. The device consists of a stainless steel chamber in which the sorted droplets are collected. In the collection chamber the collected droplets run through a capillary where a continuous fluorescence measurement is made. With a sample of fluorescent particles, the delay time is optimal when the measured fluorescence is maximal. The measuring volume is always filled with the last droplets sorted (about 3,000). With this device, the setting of the delay time can be done in a few seconds without the need for microscopical verification.

The fluorescence in the collection chamber is excited and detected via optical fibers using about 10% of the light of the existing laser from the flow cytometer and an extra photomultiplier.

Key terms: Charge pulse delay, delay time measurement, cell sorter, flow sorter, instrumentation, fluorescence

Since the introduction of the flow cytometer for biological cell analysis and sorting, the number of applications has been quite impressive. Due to numerous technical and cytochemical improvements made during the last decade, flow cytometric analysis is now a standard technique in almost every clinic and biological laboratory. The application of the droplet method for cell sorting (1,2,4), however, is still confined to the research laboratories. This is at least partly due to the fact that the cell sorter is more difficult to operate and requires skilled personnel.

One of the procedures that could benefit from simplification is the setting of the delay time between optical analysis and application of the electrical droplet charging pulse. In stream in air systems (5) this is usually done by measuring the distance between the laser illumination spot and the droplet break-off point with a built-in viewing microscope (3). By relating this distance to the distance between two droplets, the time delay can be calculated from the known droplet frequency. This method can not be used in sorters were the cell analysis is done in a flow cell. In that case, fluorescent particles must be sorted with variable time delays on a microscope slide and analyzed on a separate fluorescence microscope to determine the optimal delay time (5). Even in the stream in air sorters it is good practice to verify the time delay in this way.

In this note we describe a simple add-on to existing droplet sorters that provides an accurate time delay setting.

MATERIALS AND METHODS

The principle used for establishing the correct delay time is to sort fluorescent microspheres into a small cuvette in which a fluorescence measurement is made. If the delay time is correct, the concentration of the particles in the cuvette, and thus the fluorescence intensity, is maximal. The cuvette is constructed so that it contains only the last few thousand droplets. It is made from a single piece of stainless steel as shown in Figure 1. The sorted droplets are collected in a funnel and flow through the capillary where the fluorescence is excited and detected by optical fibers F1 and F2, respectively. The angle between the capillary and the horizontal plane is chosen such that the measuring volume is always filled and automatically refreshed.

As shown in Figure 2, the device was incorporated into a conventional droplet sorter, consisting of an air cooled argon ion laser (model 5500 ASL, Ion Laser Technology, Salt Lake City, UT), a FACS II flow cell (Becton Dickinson, Mountain View, CA) with a 100 μm nozzle operating at a droplet frequency of 25 kHz, and detection optics for light scattering and fluorescence. By inserting a glass plate in the laser path, about 10% of the laser light was coupled into fiber F1 via lens L

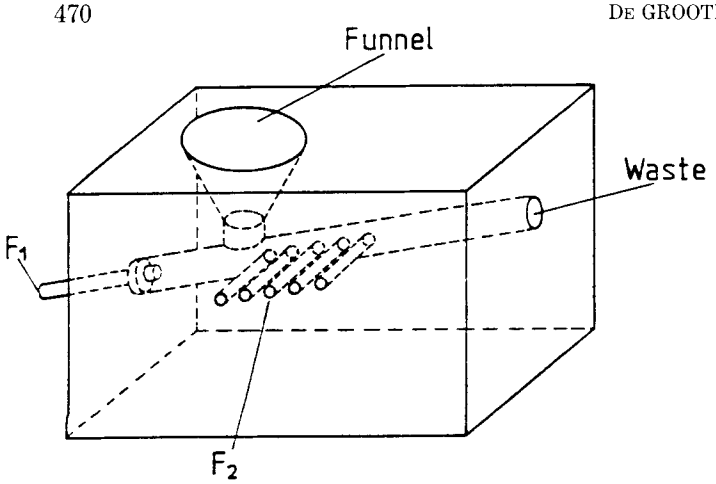

FIG. 1. Drawing of the collection chamber used for fluorescence measurements of the recently sorted droplets. The excitation light is coupled into the capillary via the optical fiber F1. The emitted fluorescence is collected with the fibers F2 mounted orthogonally towards the capillary part of the chamber. The chamber is made of stainless steel.

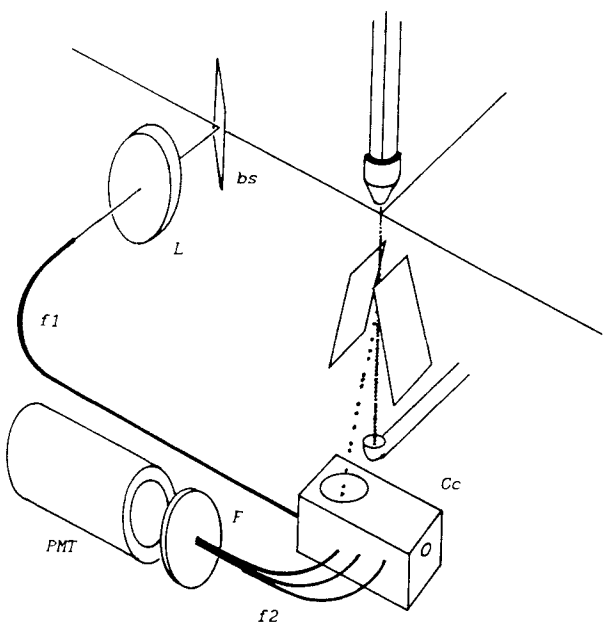

FIG. 2. Schematic drawing of the setup used to measure the time delay. A conventional sorter was used for analyses and sorting equipped with the following extras: bs, beam splitter (microscope slide); L, lens (focal length 40 mm); f1 and f2, optical fibers (inner core 600 μm); Cc, collection chamber; F, fluorescence filter; PMT, photomultiplier.

with a focal length of 40 mm. Fluorescence emission is collected by one (or more) optical fibers (F2) and detected by a photomultiplier (R928, Hamamatsu, Bridgewater, NY) provided with Schott filters (KV 520 and OG 515/3, Schott, Duryea, PA).

RESULTS

With the set-up described above the delay time setting procedure is as follows. A sample of 5.2 μm fluorescent microspheres (Polysciences, Warrington, PA) at a concentration of about 10^6 particles per ml is sorted at a rate of about 800 particles per second. The fluorescence of the collected droplets is monitored in the collection chamber. The delay time is changed slowly and adjusted for maximum fluorescence.

Figure 3 and 4 show actual recordings of this procedure. Here the delay time, expressed as the number of droplets formed during this time, was increased stepwise. In Figure 3a,b, the number of droplets deflected per sorting event was three and one, respectively. The curves clearly show that a fluorescent signal is obtained only if the correct time delay is applied. As was expected, the margin in this time is plus and minus one droplet period if three droplets are deflected. In agreement with expectation, the fluorescence maximum in Figure 3b was three times as high as that in Figure 3a: if each deflected droplet contains a fluorescent particle, the fluorescence intensity is expected to be three times higher than that obtained if only one in three droplets contain a particle.

By careful examination of Figure 3b, one can observe a small fluorescence signal at a delay of 20 droplets also. This was not due to some artifact but to the electronic sort decision method used. In our system we sum up all events measured during one droplet period. The idea is that all the particles that will later be in one droplet together determine whether the droplet has to be sorted or not. With this method it is of course essential that the starting point of this summation time correspond exactly with the starting point of a future droplet. By comparing the observed fluorescence signals in Figure 3b at a delay of 20 droplets with the maximum fluorescence signal, we estimate that the starting point of the summation was wrong by about 10% of the droplet period. In Figure 4 we deliberately used the wrong starting point. Now it is clear (see Fig. 4b) that about 35% of the particles end up in the wrong droplet.

Due to the relatively large measuring volume, variations in the fluorescence signal caused by statistical fluctuations of the number of particles in the measuring volume were avoided.

DISCUSSION

In this note we have described a simple method for determining the optimal delay time for the charging pulse in droplet sorters. The method is easy to operate and yields accurate and reliable results. Once the maximal fluorescence signal for a given standard sample of microspheres is determined, microscopic verification of the sorter performance is no longer necessary. The components needed for the device are inexpensive and easy to incorporate into existing sorters due to the flexibility of the optical fibers used.

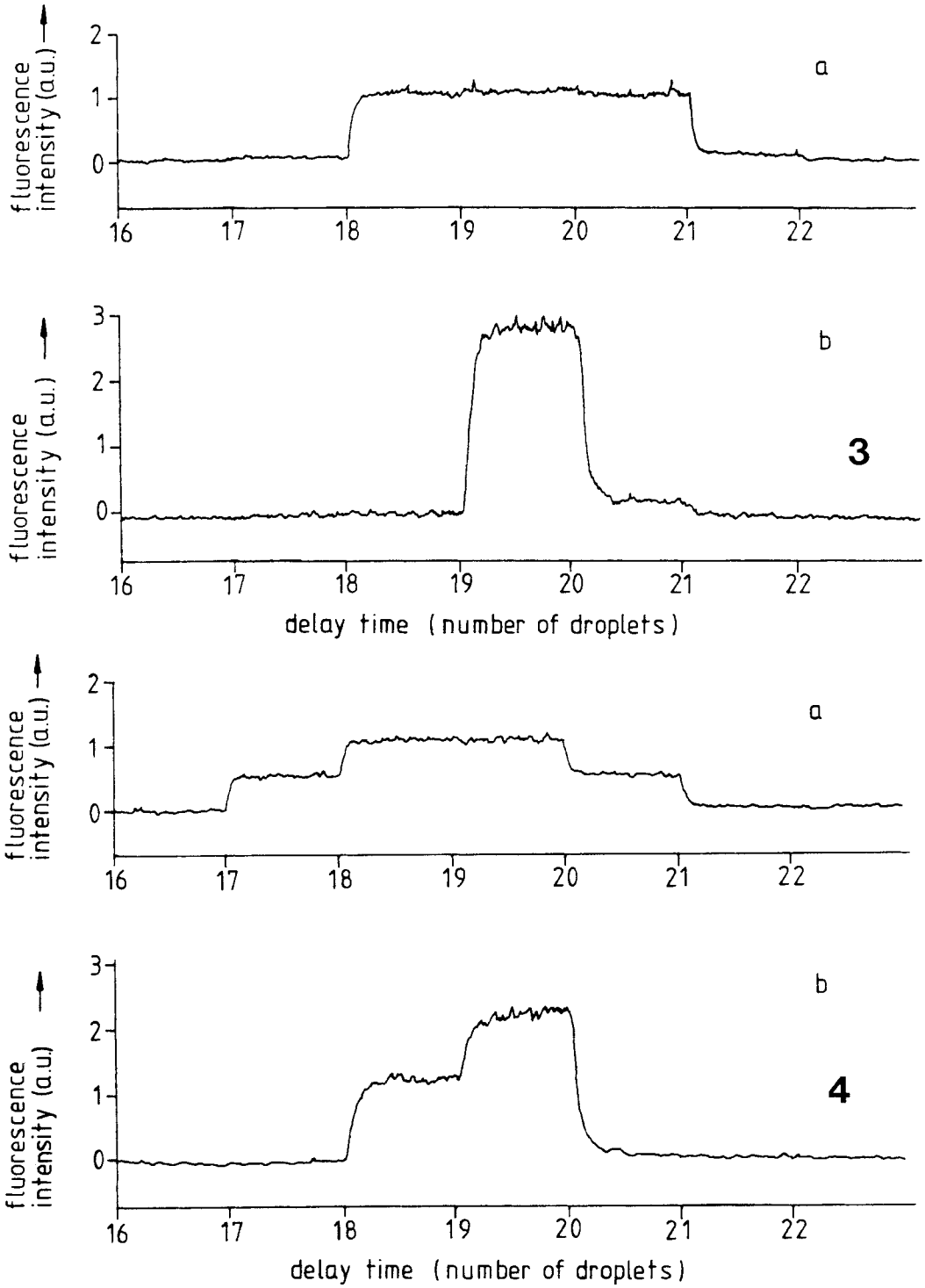

FIG. 3. Time course of the measured fluorescence signal in the deflected droplets accumulated in the collection chamber. Polystyrene microspheres (diameter 5.2 μm) were sorted. During the measurement the time delay was increased step wise every 90 s. The numbers correspond to the time delay expressed in the number of droplets formed during the delay. **Trace a:** Three droplets deflected per sort event. **Trace b:** One droplet deflected per sort event. (Two F2 fibers were used in these recordings).

FIG. 4. Time course of the fluorescence measured in the collection chamber when three droplets **(trace a)** or one droplet **(trace b)** are deflected per sort event, as a function of the delay time. The experimental conditions were the same as in Figure 3 except that the electronic delay time was deliberately maladjusted by about one-third of a droplet period.

In a droplet sorter, usually three droplets per sort event are sorted. The advantage of this is that the time delay is not so critical (see Fig. 3a). The price for this is that the maximum achievable sorting rate is decreased by a factor of three. If only a single droplet per sorting event is deflected, precise setting of the time delay is essential. With the continuous monitor described here, this is easily achieved. Thus the monitor described here can be of great help when sorting with high purity, high efficiency, and high speed is required. Sorting only one droplet per sort event increases the sorting rate by a factor of three, whereas the purity and efficiency can be maintained by the optimal delay setting. In principle the method used should also be applicable to control a long sort experiment. A change in the instrument setting changes the amount of fluorescence in the sorted droplets. Since most sorters enable the selection of two populations, in cases where only one resultant population is desired, fluorescent beads can be added to the samples as a tracer, enabling continuous monitoring of long sorts. Although in its present form the method is not sensitive enough for practical immunofluorescence measurements, increasing the laser power and the light collection efficiency could make these possible.

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