A microfabricated fluorescence-activated cell sorter

Anne Y. Fu[1,2], Charles Spence[1], Axel Scherer[1], Frances H. Arnold[2], and Stephen R. Quake[1]*

[1]Department of Applied Physics, [2]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125.
**Corresponding author (e-mail: quake@caltech.edu).*

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We have demonstrated a disposable microfabricated fluorescence-activated cell sorter (μFACS) for sorting various biological entities. Compared with conventional FACS machines, the μFACS provides higher sensitivity, no cross-contamination, and lower cost. We have used μFACS chips to obtain substantial enrichment of micron-sized fluorescent bead populations of differing colors. Furthermore, we have separated *Escherichia coli* cells expressing green fluorescent protein from a background of nonfluorescent *E. coli* cells and shown that the bacteria are viable after extraction from the sorting device. These sorters can function as stand-alone devices or as components of an integrated microanalytical chip.

Keywords: microchips, cell sorting, disposable chip, flow cytometry

Conventional fluorescence-activated cell sorters (FACSs) are widely used to study eukaryotic cell populations. Although they provide impressively efficient sorting, they are costly ($250,000), mechanically complex, and require trained personnel for operation and maintenance. Inexpensive devices that rapidly sort live cells, particles, and even single molecules would greatly facilitate screening of combinatorial chemistry libraries or cell populations during in vitro molecular evolution. Moreover, such devices would have wide applications in clinical medicine and basic biological and materials research.

All modern conventional flow cell sorters are designed to have a flow chamber with a nozzle and are based on the principle of hydrodynamic focusing with the sheath flow[1–5]. In addition, most sorting instruments combine the technology of ink-jet printing and electrostatic deflection to achieve droplet generation and high sorting rates[6,7]. However, this mechanism is delicate and many failures of the instrument can result from problems in the flow chamber. For example, clogging of the orifice and particle adsorption and contamination in the tubing can cause turbulent flow in the jet stream, inducing variation in illumination and detection. Sample carryover can occur during consecutive runs when remnants of previous samples backflush into the new sample stream, and sterilizing the system between runs is time-consuming and results in machine downtime. Furthermore, cells passing through the orifice may perturb droplet formation: Larger cells can change the droplet size, nonspherical cells tend to align with the long axis parallel to the flow axis, and deformable cells may elongate in the direction of the flow[1,2]. Such perturbations in droplet formation can introduce variation in the time from the analysis to the actual sorting event. Finally, a number of technical problems make it difficult to generate identically charged droplets, in turn increasing the deflection error.

Replacing the conventional flow chamber in FACS with microfabricated devices potentially can allow more sensitive optical detection, easier mechanical setup, and innovative sorting schemes. Other groups have demonstrated that cells, particles, and reagents can be manipulated in microfluidic devices by pressure, dielectrophoresis, and electro-osmosis[8–11]. In previous work, we described a microfabricated flow cytometer capable of detecting single DNA molecules[12]. We now have constructed a complete microfabricated fluorescence-activated cell sorting (μFACS) device and demonstrated its effectiveness for sorting micron-sized latex beads and bacterial cells.

This disposable sorting device is fabricated using a micromachining technology called "soft lithography,"[13] which enables the design of inexpensive and flexible miniaturized fluidic devices. Microfabrication permits integration of cell sorting with other techniques such as PCR[14], microfabricated total analysis systems[15], and DNA chip hybridization[16], and allows novel sorting algorithms that are not possible in conventional cell sorters. Another advantage is that multiple cell sorters can be fabricated in parallel on a single chip, allowing increased throughput or successive enrichments of a sample.

Results and discussion

The μFACS device is a silicone elastomer chip with three channels joined at a T-shaped junction (Fig. 1). The channels are sealed with a glass coverslip. A buffer solution is introduced at the input channel and fills the device by capillary action. The pressure is equalized by adding buffer to the two output ports and then adding a sample containing the cells to the input port. The cells are manipulated with electro-osmotic flow, which is controlled by three platinum electrodes at the input and output wells. The chip is mounted on an inverted optical microscope, and fluorescence is excited near the T-shaped junction with a focused laser beam. The fluorescent emission is collected by the microscope and measured with a photomultiplier tube (PMT). A computer digitizes the PMT signal and controls the flow by the electro-osmotic potentials (Fig. 2).

The standard "forward" sorting algorithm consists of running the cells from the input channel to the waste channel until a cell's fluorescence is above a preset threshold, at which point the voltages are temporarily changed to divert the cell to the collection channel (Fig. 3). Extending the system to include detection of multiple-color fluorescence and light scattering, as are used in conventional FACS machines, is straightforward. An advantage of μFACS is the small detection volume, typically approximately 250 femtoliters, which greatly reduces background fluorescence from cell suspension and chamber material.

Different algorithms for sorting in the microfluidic device can be implemented by computer. As an example, consider a pressure-switched scheme instead of electro-osmotic flow. With the latter, switching is virtually instantaneous and throughput is limited by the highest voltage that can be applied to the sorter (which also affects the run time through ion depletion effects). A pressure-

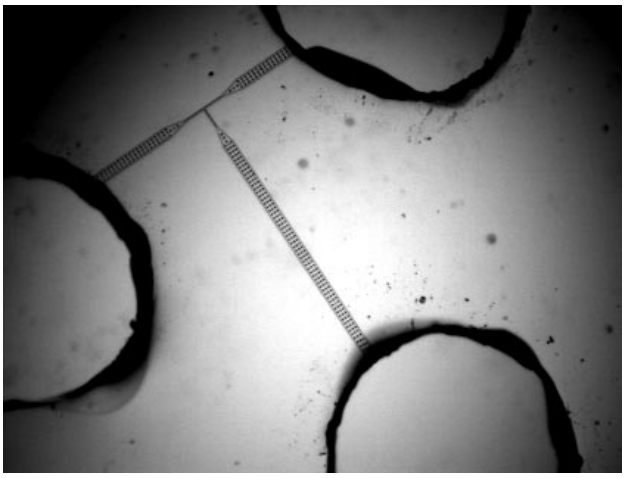

Figure 1. Optical micrograph of the μFACS device. The device shown has channels that are 100 μm wide at the wells, narrowing to 3 μm at the sorting junction. The channel depth is 4 μm, and the wells are 2 mm in diameter.

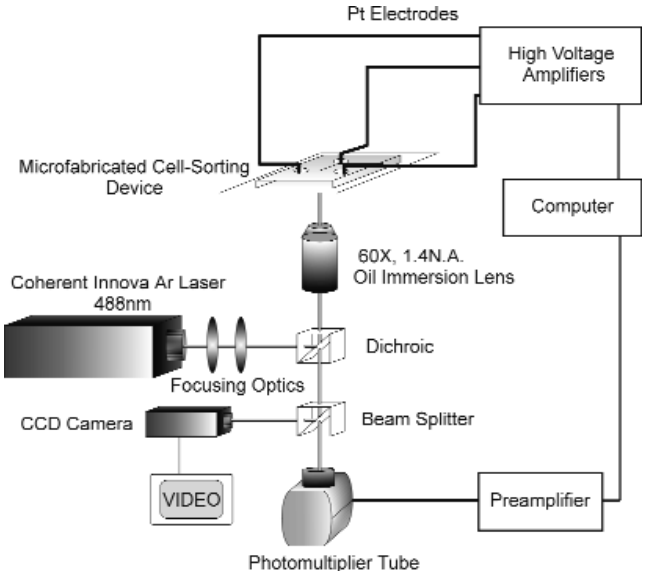

Figure 2. Schematic diagram of the cell sorting apparatus. See Experimental Protocol for details.

switched scheme does not require high voltages and is more robust for longer runs.

Mechanical compliance in the system is likely to cause the fluid switching speed to become rate limiting with the forward sorting program. Because the fluid is at low Reynolds number and is completely reversible, it is possible when trying to separate rare cells to implement a sorting algorithm that is not limited by the intrinsic switching speed of the device. The cells flow at the highest possible static (non-switching) speed from the input to the waste. When an interesting cell is detected, the flow is stopped. By the time the flow stops, the cell is past the junction and part way down the waste channel. The system is then run backward at a slow (switchable) speed from waste to input, and the cell is switched to the collection channel when it passes through the detection region. At that point the cell is saved, and the device can be run at high speed in the forward direction again (Fig. 3). This reversible sorting method is not possible with standard FACS machines and should be particularly useful for identifying rare cells or making multiple measurements of a single cell.

The use of μFACS for forward and reverse sorting with electroosmotic flow was demonstrated with fluorescent beads of different emission wavelengths in different ratios and up to 33,000 beads per hour throughput (Table 1). Extra reservoir wells were incorporated on the outer side of the three wells in order to avoid ion depletion, and platinum electrodes (with the ground electrode in the input well) were inserted into the reservoir wells. The collection wells were filled with buffer, and a mixture of red and blue fluorescent beads was injected into the input well in aliquots of 10–30 μl. The optical filter in front of the PMT passed only red fluorescence, allowing selective sorting of red beads. Sorting can be performed for as long as 3 h with occasional readjustment of the voltage settings. The coefficient of variation in bead intensity was measured to be 1–3%, depending on the depth of the channel and the surface treatment of the elastomer.

A single pass through the μFACS in the forward mode produced a highly enriched sample of red beads (Table 1). Whereas the initial concentration of red beads was 10%, the output well held 84% red beads, whereas the waste had <1%. Similar results were obtained when running in reverse sorting mode when the initial concentration of red beads was lowered to 1%. Run times varied from 10 min to 3 h. With both forward and reverse sorting, enrichments of 80-fold to 96-fold were obtained in single runs, in which the enrichment is defined by the increase in the fractional concentration of red beads.

We have also demonstrated that the device can sort living *Escherichia coli* cells, and that the cells are viable after sorting. Different ratios of wild type to green fluorescent protein (GFP)-expressing *E. coli* cells were introduced into the input well (volume ranges from 10 to 30 μl of sample); the collection wells were filled with 10–30 μl of buffer with 10^{-5} M SDS. After the three platinum electrodes were inserted into the wells (with the ground electrode in the input well), the voltages were set for forward sorting. After 2 h of sorting, cells were collected through with a pipette and streaked onto Luria–Bertani (LB) agar plates and incubated overnight at 37°C for colony counting. We achieved enrichments of 30-fold with yields of 20%, where the yield is defined by the number of colonies on the plate divided by the number of positive fluorescence events detected in the device. Recovery of viable cells was relatively constant at 20% in electric fields up to about 100 V/cm, corresponding to velocities of about 1–3 mm/s (Table 1).

Table 1. Results of sorting red from blue fluorescent beads (forward mode and reverse mode) and of sorting GFP-expressing HB101 *E. coli* from wild-type HB101 *E. coli* (forward mode)[a]

	Input well		Collection well		Waste well	
Bead color	Blue	Red	Blue	Red	Blue	Red
Forward-mode bead sorting	0.925	0.074	0.160	0.840	0.998	0.002
Reverse-mode bead sorting	0.988	0.012	0.043	0.957	0.999	0.001
	Wt	GFP	Wt	GFP	Wt	GFP
HB101 *E. coli* cell sorting	0.992	0.008	0.693	0.307	0.992	0.008

[a]For the forward mode, after running for 22 min the collection channel had a sample of red beads that had been enriched by 8.4 times from an initial blue:red bead ratio of 10:1. For the reverse mode, after 6 min of sorting, red beads in the collection channel had been enriched 80 times from an initial blue:red bead ratio of 100:1. The throughput was ~10 beads/s. The initial ratio of wild type-(Wt) to green fluorescent protein (GFP)-expressing HB101 *E. coli* cells was 100:1. After 2 h of sorting, cells recovered from the collection well were enriched 30 times (approximately 120,000 cells sorted). Numbers in the table represent the fraction of different beads or cells in each well.

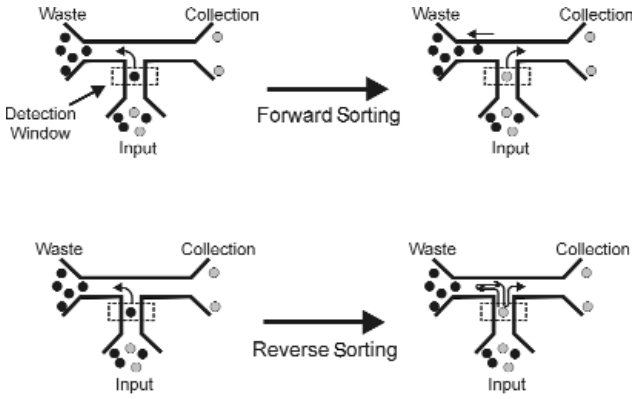

Figure 3. A sketch of the algorithms for the forward sorting and reverse sorting is shown with the schematic of the T-shaped junction. Top: With the forward-sorting algorithm, the fluid flow is switched as fluorescence is detected so that red beads are sent directly to the collection channel. Bottom: The reverse-sorting algorithm allows rare-event capture at a rate independent of the switching speed of the device. Fluid flow is established at a high rate from the input well to the waste channel. After event detection, the fluid flow is halted and reversed until the bead is detected a second time. It is then directed at a slower speed to the collection channel.

Our μFACS system offers several advantages over traditional sheath flow methods. Because the channels in the device can be made with micron dimensions, the volume of the interaction region can be precisely controlled, and there is no need for hydrodynamic focusing. The planar geometry of the devices allows the use of high numerical aperture (NA) optics, increasing the sensitivity of the detection system. We previously showed that microfluidic analytical systems with a similar geometry are sensitive enough to identify a single 2 kbp molecule of DNA[12]. As fluid flows continuously through the system, there is no need for droplet formation and a host of challenging technical issues can be sidestepped. Furthermore, no aerosol is formed because the system is entirely self-contained, allowing relatively safe sorting of biohazardous material. The disposability of the sorting devices obviates the need for cleaning and sterilizing the instrument and prevents cross-contamination between samples.

The throughput of 20 cells/s is considerably slower than conventional FACS machines, but there is reason to believe the throughput can be augmented by increasing the electric field by up to a factor of six (ref. 10). Throughput also can be improved in future systems either through parallel device fabrication or with a pressure-driven switching scheme. It should also be possible to sort eukaryotic cells with μFACS, given that others have shown that eukaryotic cells can be manipulated electro-osmotically in microfabricated devices[10] and are compatible with the elastomeric surface chemistry[11] (Robert H. Austin, personal communication).

A working μFACS system can be assembled for approximately $15,000. Most of this amount represents the cost of the external optics and detectors used to read out the chip, since the cost of the chip itself is negligible. Considerable cost savings can potentially be realized by fabricating the detectors and optical filters directly on the chip. We believe that this will be an important component of future integrated biomedical chip-based systems.

Experimental protocol

Microfabrication. The chip was fabricated as described[12]. Briefly, standard micromachining techniques were used to create a negative master mold out of a silicon wafer. The silicone elastomer was poured on the wafer and allowed to cure for 2 h at 80°C. The resulting device could be peeled off of the wafer and bonded hermetically to glass. It was rendered hydrophilic by boiling in HCl (pH 2.7, 0.01% in water) at 60°C for 40 min. The master wafer can be reused indefinitely.

System setup. The cell-sorting device is mounted on an inverted microscope (Zeiss Axiovert 35; Carl Zeiss Inc., Thornwood, NY) with an oil immersion objective (Plan Apo Chromat 60×, 1.4 NA; Olympus America Inc., Melville, NY). Epifluorescent excitation was provided by an argon ion laser (Innova 70, Coherent Inc., Santa Clara, CA) for cells and a 100 W mercury lamp for beads. Fluorescence was collected with the same objective and projected onto the cathode of a Hamamatsu R928 (Hamamatsu, Bridgewater, NJ) PMT with custom current-to-voltage amplifier. Part of the light can be directed onto a charge-coupled device (CCD) camera for imaging. The detection region is approximately 5–10 μm below the T-shaped junction and has a window of about 15 × 5 μm in dimension. The window is implemented with a Zeiss adjustable slit. Cells or particles can be directed to either side of the T channels depending on the voltage-potential settings. The voltages on the electrodes are provided by a pair of Apex PA42 HV operational amplifiers (Apex Microtechnology, Tuscon, AZ) powered by 150 V Acopian power supplies. The third electrode is ground. The PMT signal is digitized by the personal computer, which also controls the high voltage settings via a National Instruments (Austin, TX) Lab PC1200 card.

Preparation of beads. Red and blue fluorescent beads (1 μm diameter, Interfacial Dynamics Corporation, Portland, OR) were suspended in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4) with 10% BSA (1 g/L) and 0.5% Tween 20 in a 10:1 blue:red ratio and overall concentration of 1.5%. Fluorescence of the beads was excited by 100 W mercury lamp with 488DF20 optical filter. A 630DF30 optical filter (Chroma Technology Corp., Brattleboro, VT) was used to select the red fluorescent emission. The μFACS device had 3 × 4 μm channels. A 100:1 blue:red ratio was used for reverse sorting.

Preparation of *E. coli* cells for sorting. The *E. coli* cells (HB101) expressing GFP were grown at 30°C for 12 h in LB liquid medium containing ampicillin (one colony inoculated into 3 ml medium containing 50 μg/ml of ampicillin). Wild-type *E. coli* HB101 cells were incubated for 12 h in LB-only medium. After incubation, HB101 and GFP-expressing HB101 *E. coli* cells were resuspended into PBS (ionic strength = 0.021) three times and stored at 4°C for sorting. Immediately before sorting, the cells were resuspended again into phosphate buffer (4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4) containing 10^{-5} M SDS and diluted to a concentration of 10^9 cells/ml. The cells were filtered through a 5 μm syringe filter (Millipore Bioscience Inc., Bedford, MA) for elimination of any elongated cells. A μFACS device with 10 × 4 μm channels was used. Fluorescence was excited by the 488 nm line of an argon ion laser (6mW into the objective), Coherent Innova 70 (Laser Innovations), and the emitted fluorescence was filtered with a 535DF20 filter (Chroma).

Acknowledgments

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