

# FLOW CYTOMETRY ON MICROFLUIDIC DEVICES

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## ABSTRACT

Flow cytometry was demonstrated on a microfabricated fluidic device. The channels were coated to prevent cell adhesion, and the cells were transported electrophoretically by applying potentials to the fluid reservoirs. The sample stream was spatially confined in two dimensions at the cross intersection to conduct coincident light scattering and fluorescence detection. An *Escherichia coli* sample was labeled on-chip with a membrane permeable nucleic acid stain Syto 15 and counted.

## INTRODUCTION

Interest in microfabricated instrumentation for chemical sensing and analysis has grown exponentially over the past decade primarily because these miniature instruments may provide information rapidly and reliably at low cost. Microfabricated fluidic devices (microchips) constructed on planar substrates are advantageous for manipulating small sample volumes, rapidly processing materials, and integrating sample pretreatment and separation strategies. The dexterity with which materials can be manipulated and the ability to machine structures with interconnecting channels with essentially zero dead volume contribute to the high performance of these devices. To carry out a complete assay, functional elements can be serially integrated on these devices and include filters, valves, pumps, mixers, reactors, separators, cytometers, and detectors. Coupling these elements together under computer control will enable the development of a wide range of microchip-based assays. One area of particular interest is the analysis of cells and cell populations, and a rapid screening technique for such assays is flow cytometry [1].

A few examples of cell manipulations on microfluidic devices have appeared where hydrodynamic [2,3] and electroosmotic [4,5] flows have been used to transport and sort cells on microchips. We are developing microfabricated fluidic devices for flow cytometry that incorporate electrokinetic focusing to spatially confine fluids [6] and particles [7]. The particles in this narrowed sample stream are then detected using light scattering and/or fluorescence detection. In this proceedings, we describe the operation and performance of a microfluidic device for cytometry that characterizes an *E. coli* sample labeled with a membrane permeable stain Syto 15.

## EXPERIMENTAL

*Fabrication.* Two microchips designs similar to Figure 1 were transferred by UV exposure from the photomask (HTA Photomask) to glass substrates coated with chromium and positive photoresist (HOYA). After developing the photoresist, the

chromium film was etched ( $\text{CeSO}_4/\text{HNO}_3$ ), then the channels were etched into the substrate in a dilute, circulated  $\text{HF}/\text{NH}_4\text{F}$  bath. Channel access holes 2-mm in diameter were drilled in the cover plate. To form the closed network of channels, the cover plate was bonded to the substrate over the etched channels by hydrolyzing both surfaces, bringing the cover plate into contact with the substrate, and annealing at  $500^\circ\text{C}$ . Cylindrical glass reservoirs were then affixed on the cover plate using epoxy. The channels were  $50\ \mu\text{m}$  wide at the cross intersection. The channel widths were measured at half-depth using a stylus-based profiler. Before analyzing the sample, the microchip was sequentially rinsed for 15 minutes with 1.0 M NaOH, water, Run Buffer (CE-SDS Protein Kit, BioRad), water, and phosphate buffered saline (PBS). After each set of experiments, the microchips were rinsed in the same manner with 50 mM SDS and water.

*Operation.* Four high-voltage power supplies (UltraVolt) were connected to the sample, focus 1, focus 2, and waste reservoirs and independently controlled through a multifunction I/O card (PCI-MIO-16XE-50, National Instruments). Sample was continuously infused into the focusing chamber. The sample stream width was controlled by varying the potentials applied to the focus reservoirs relative to the sample reservoir.

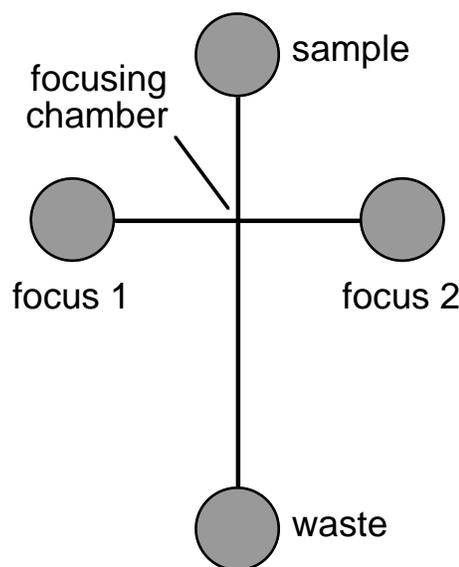


Figure 1. Schematic of microchip for flow cytometry.

**Detection.** For coincidence measurements, light scattering and laser-induced fluorescence signals were acquired simultaneously. The laser beam ( $\text{Ar}^+$ , 514 nm, 10 mW, Innova 300 FReD, Coherent) was brought to a focus 50  $\mu\text{m}$  downstream of the focusing chamber. The incident angle of excitation was  $45^\circ$ . The scatter and fluorescence signals were collected simultaneously from the microchip using a 20x microscope objective (N.A. 0.4, Edmund Scientific). The scatter and fluorescence signals were spatially filtered (200  $\mu\text{m}$  x 200  $\mu\text{m}$  square), split using a dichroic filter (540 nm, Omega Optical), and measured by two photomultiplier tubes (PMT, 77348, Oriel). The fluorescence signal was again spectrally filtered using a bandpass filter centered at 560 nm with a 40 nm bandwidth (Omega Optical). The signals from the PMTs were then amplified (428-MAN, Keithley) and digitized at 1 kHz with the same multifunction I/O card and computer used for the voltage control. Data were collected in 60-second blocks. Statistical analysis from the Syto 15 stained cells was based on eight, one-minute runs.

**Cells.** All experiments were performed with the non-pathogenic *E. coli* strain Y1090. The cells are rod shaped, between 0.7 and 1.5  $\mu\text{m}$  long, depending on their stage in cell division. A fresh culture was used for every experiment. A single colony, taken from an agar plate, was added to 2 ml of cell culture media with 50  $\mu\text{g}/\text{ml}$  ampicillin (International Biotechnologies) and incubated for four to six hours. The culture was resuspended in water, and the cell concentration was measured at 600 nm with a spectrophotometer. A small volume of the suspension was added to diluted PBS (pH 7.5, J.T. Baker) in the sample reservoir of the microchip with the fluorescent dye prior to the experiment. The cell concentration in the sample reservoir was approximately  $1.25 \times 10^8$  cells/ml in a total volume of 40  $\mu\text{l}$ . The Syto 15 dye (4  $\mu\text{M}$ , Molecular Probes) was incubated with the cells in the sample reservoir for 10 minutes prior to the experiment. The focus 1,

focus 2, and waste reservoirs were each filled with 40  $\mu\text{l}$  of PBS buffer.

## RESULTS AND DISCUSSION

To prevent cell adhesion, the channel walls of the microchip in Figure 1 were coated with Run Buffer (BioRad). This product was effective in preventing adhesion, was hydrophilic, and had only to be flushed through the channel manifold. After the microchip was prepared, operation for several hours without noticeable degradation in cell flow was possible, but operation was not tested for periods over six hours. The Run Buffer, however, minimized electroosmotic flow, and as a result, the cells had to be electrophoretically confined. Focusing of cells is pictured in Figure 2 and was accomplished by applying 268 V to the sample reservoir, 43 V to the focus 1 reservoir, 0 V to the focus 2 reservoir, and 355 V to the waste reservoir. The cells were negatively charged in the PBS buffer and flowed from the sample to the waste reservoir in the presence of an electric field. At the cross intersection, the stream of cells was narrowed in two dimensions enabling single cell interrogation at the detection point.

Figure 3 shows the light scattering (bottom) and fluorescence (top) signals collected from Syto 15 labeled cells. A potential of 0 V was applied to the sample reservoir, 10 V to the focus 1 and focus 2 reservoirs, and 560 V to the waste reservoir. Each peak represents a single cell passing through the detection zone defined by the spatial filter. The correlation between the scatter and fluorescence channels was almost unity for these experiments. The fluorescence channel had a lower signal-to-noise ratio than the scatter channel, and consequently, some fluorescence peaks were not counted by the peak finding algorithm due to insufficient signal. The scatter peaks were nearly uniform in width and height, but a wide variation in fluorescence intensities was observed. The

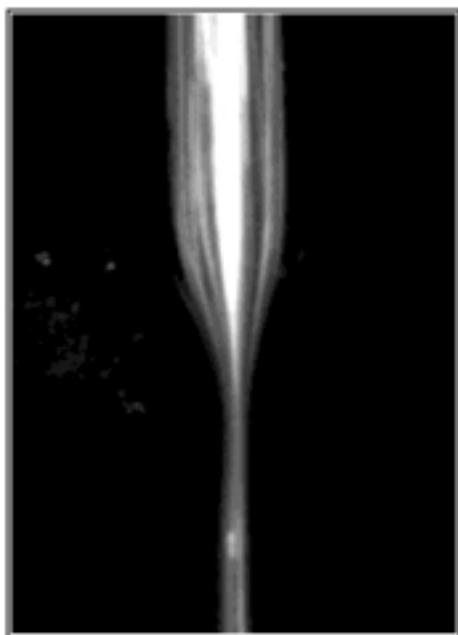


Figure 2. Fluorescence CCD image (5 s exposure) of electrophoretically focused cells labeled with Syto 15.

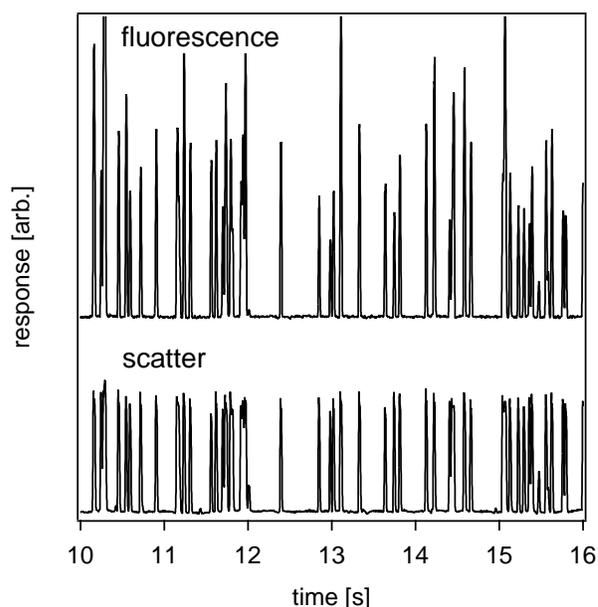


Figure 3. Light scattering (bottom) and fluorescence (top) signals from an *E. coli* sample stained with Syto 15.

variability in fluorescence from cells containing the Syto 15 stain was probably dependent on cell age and nucleic acid content. Also, with this configuration counting frequencies up to 14 Hz were recorded. Higher count rates were not possible here because at higher field strengths and cell concentrations, the cells started to aggregate hindering single cell detection.

In conclusion, these experiments demonstrate the potential of incorporating cytometry into a microfabricated fluidic device. Other assays to be presented include cell viability studies and immunoassays using a similar device and sample. Methods of increasing sample throughput, such as using higher operating flow rates and microchips with modified channel geometries, are being explored to achieve 100 to 1000 Hz counting rates per channel. Throughput can be further enhanced by multiplexing these cytometry elements in an array configuration [6].

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