RAPID CELLULAR ASSAYS ON MICROFABRICATED FLUIDIC DEVICES

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Abstract
Functional elements are being developed to load cells with fluorescent markers, perform cytometry, lyse cells, and separate the lysate contents on a microfluidic device.

Keywords: cancer, cellular assay, kinase, lysate

1. Introduction

Early diagnosis of cancer is the best prognostic indicator of successful treatment. Cancerous tissue, however, often goes undetected for several years until some clinical symptom manifests itself. To provide earlier diagnosis and to improve our understanding of oncogenesis, high-throughput, sensitive, and inexpensive cellular assays need to be developed to detect the rare mutations in cells that are responsible for tumor formation and eventual metastasis. Microfabricated fluidic devices potentially provide a convenient platform for such assays. While these assays may require multiple chemical processing steps, all of the steps can potentially be integrated into a small inexpensive device. These steps include cytometry for the examination of a large number of cells, microfluidic switches to isolate rare cells of interest, cell lysis, and the separation, detection and identification of the cellular lysate components.

2. Experimental

A simple cross chip design was used to test several functional elements. The chips were fabricated on soda-lime glass using standard photolithographic, wet chemical etching, and cover plate bonding techniques. The cells were moved using either applied electric fields or hydrodynamically induced flow generated by a syringe pump. An inverted microscope with an epi-illumination attachment and a CCD camera were used to image the cells as they flowed through the chips. A single-point setup was used for the cytometry and for detecting the contents of lysed cells.

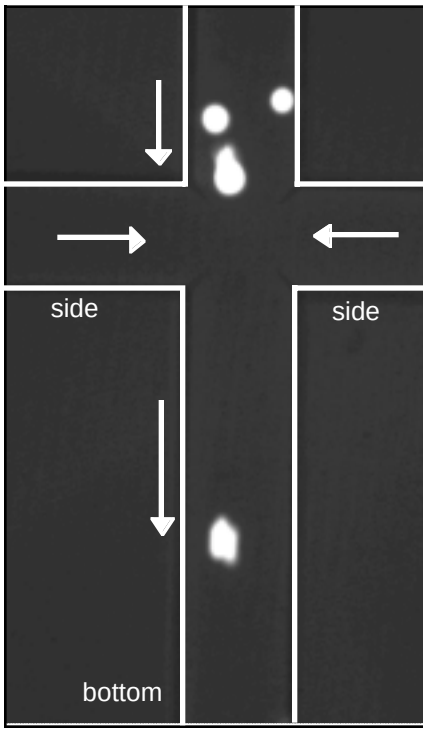

Figure 1. Image of microchip channel containing cells loaded on-chip with calcein AM. The arrows designate the direction of fluid flow.

3. Results and Discussion

Figure 1 is an image of a microchannel with Jurkat cells flowing through it. The Jurkat cells were labeled on-chip using calcein AM. The cells were brought hydrodynamically to a cross intersection where a combination of chemical and electrical lysis was performed. The chemical lysate was added through the side channels and the electric field was applied between the cross and the bottom channel. The cells were quickly lysed (< 66 ms); a necessary requirement for accurate analysis of many intracellular species. Downstream in the analyte channel the released calcein AM was detected. A coincidence detection method was used where both forward laser light scatter and fluorescence were detected (**Figure 2**). In the event of successful lysis little or no signal was seen in the scatter channel.

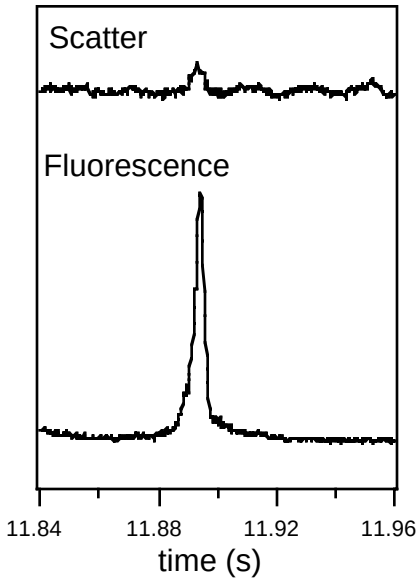

Figure 2. Single-point coincidence detection of calcein AM plume released from cell after lysis. The detection is downstream from the lysing chamber.

We have also improved significantly our ability to perform cytometry on a microfluidic device. **Figure 3** shows the coincident forward laser light scattering and fluorescent detection of *E. coli* labeled on chip with propidium iodide. The cell throughput was 85 Hz. This chip was coated with poly(dimethyl-acrylamide) to prevent cell adhesion. The cells were moved through the chip based on their electrophoretic mobility because the coating essentially eliminated electroosmosis.

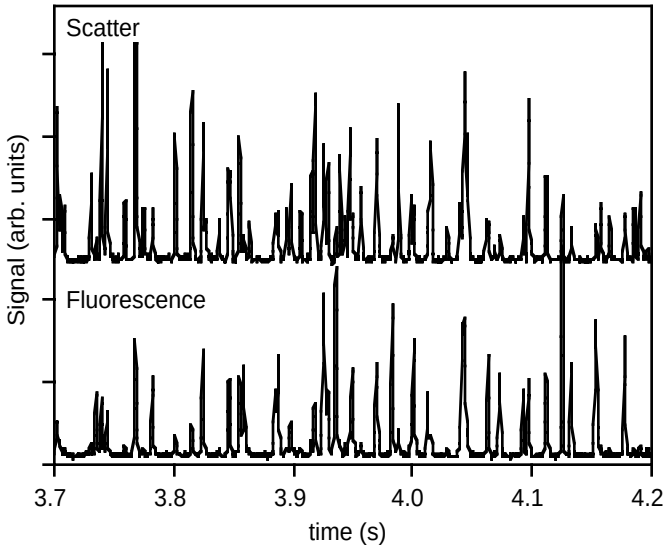

Figure 3. Single-point coincidence detection of *E. coli* labeled with propidium iodide. Each peak represents an individual cell.

Acknowledgements

Oak Ridge National Laboratory is managed and operated by UT-Battelle, LLC under contract DE-AC05-00OR22725 with the U.S. Department of Energy.