

MICROMACHINED BROADBAND RF CYTOMETER FOR HIGH-THROUGHPUT ANALYSIS OF MAMMALIAN CELLS

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ABSTRACT

We have microfabricated a continuous-flow, high-bandwidth Coulter counter that uses a radiofrequency (RF) probe to measure RF reflectance from biological cells in the microfluidic channel. This scheme eliminates the trade-off between detection sensitivity and throughput, which exists in previous approaches. Using this technique, we have demonstrated the fastest microfluidic cytometer reported to date, which operates at 240 MHz and has intrinsic detection rates near 10 MHz. We also present real-time detection data on human breast tumor cells.

Keywords: Coulter counter, cell impedance, cytometer, MEMS

1. INTRODUCTION

The Coulter counter¹ is an essential tool in hematology and oncology, where the size and concentrations of blood and tumor cells are analyzed in real time by the measurement of impedance changes across a narrow constriction. The miniaturization of the Coulter counter and its integration into inexpensive, disposable microfluidic systems presents a path to eliminate sample cross contamination. Previous approaches to microfabricated Coulter counters faced a major limitation, in that the high throughput (number of cells measured per second) is achieved at the cost of measurement frequency and sensitivity²⁻⁵: The high electrical impedance of the microfluidic channel filled with ionic solution, combined with the parasitic capacitance that shunts detection current, severely degrades the detection sensitivity at high frequencies, thereby limiting the detection rates and measurement bandwidth. On the other hand, the ionic double-layer capacitance on electrodes hinders the sensing of small resistance changes at low frequencies⁴. In order to address this problem, we implement a novel radiofrequency (RF) detection technique⁶ through which we extend the measurement frequency beyond 240 MHz, far above previous reports²⁻⁵, without sacrificing sensitivity.

2. EXPERIMENTS AND RESULTS

An RF resonance detection scheme⁶ was employed to monitor the RF reflection from the microfluidic device as a function of time and fluid content. A cell entering the detection region reflects a higher fraction of the incident RF power than the ionic medium, which is subsequently sensed by a tuned RF receiver. Using this technique, we can significantly reduce the impact of the parasitic capacitance, and thus extend the detection bandwidth without compromising the sensitivity.

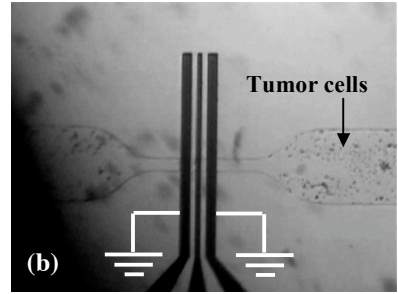
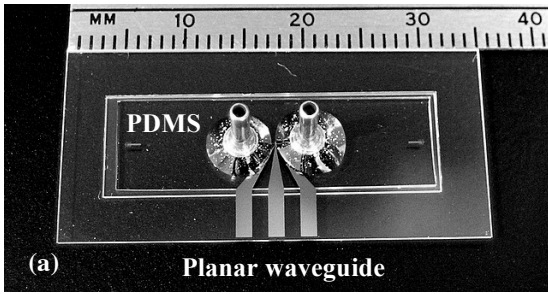


Figure 1: (a) Micromachined RF cytometer. Ti-Au coplanar waveguide carries RF signals in and out of the detection region. (b) Top-down view of the $50 \times 50 \mu\text{m}^2$ channel and the detection region. Human breast tumor cells (ZR-75-1; $10 \mu\text{m}$) are shown in the channel.

Figure 1 shows the micromachined cytometer with fluidic and electrical outlets. A sub-miniature coaxial connector carries RF signals to a Ti-Au coplanar waveguide patterned on a glass substrate. A fluidic channel with a $50 \times 50 \mu\text{m}^2$ cross-section is defined by PDMS molding⁷. The gap between the center electrode and two adjacent ground electrodes defines the active sensing region, which is approximately $50 \mu\text{m}$ wide and $60 \mu\text{m}$ long with $20 \mu\text{m}$ of separation between the electrodes.

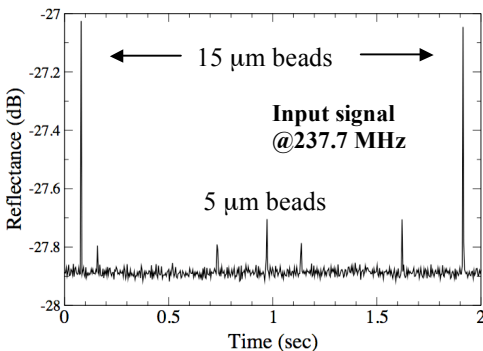


Figure 2: Real-time reflectance measurement from polystyrene beads in a PBS buffer with 0.168g/mL sucrose.

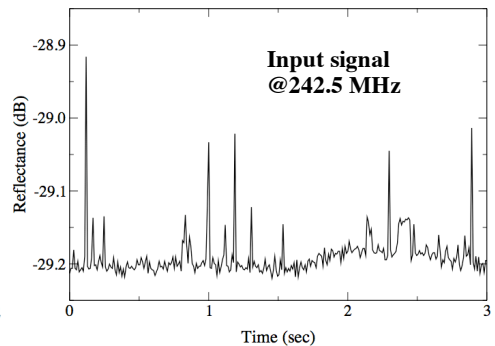


Figure 3: Reflectance measurement from breast tumor cells (ZR-75-1) in a PBS buffer.

The reflectance signal (S_{11}) from a mixture of $5 \mu\text{m}$ and $15 \mu\text{m}$ polystyrene beads in the $50 \times 50 \mu\text{m}^2$ channel filled with PBS was measured (Figure 2). In this experiment, the input signal was at 237.7MHz and the buffer conductivity was $\sigma = 1.4 \text{S/m}$ with 0.168g/mL sucrose. The data demonstrates the ability to perform size analysis based on signal amplitudes; signals from $5 \mu\text{m}$ and $15 \mu\text{m}$ beads are clearly distinguishable, and were confirmed by simultaneous optical monitoring.

Real-time detection of human breast tumor cells (ZR-75-1) in PBS flowing at $20 \mu\text{L/hr}$ was measured (Figure 3). Residual variations in reflectance correspond to aggregates of multiple tumor cells. Due to the high sensitivity of our device, the use of narrow constriction was not necessary which helped to reduce clogging and high pressures in the channel.

A high-resolution velocity measurement of 15 μm polystyrene beads was performed (Figure 4). Two distinct peaks were observed as the bead crosses the two detection regions formed by the center and the adjacent ground electrodes (40 μm peak-to-peak separation). The velocity of the bead was determined by measuring the time to traverse 40 μm separation. At flow rates of 10 $\mu\text{L/hr}$ and 30 $\mu\text{L/hr}$, the velocity of the bead was determined to be of 2.96 mm/s and 6.54 mm/s, respectively. The discrepancy from the theoretical maximum velocity is due to the uncertainty in the bead position within the channel.

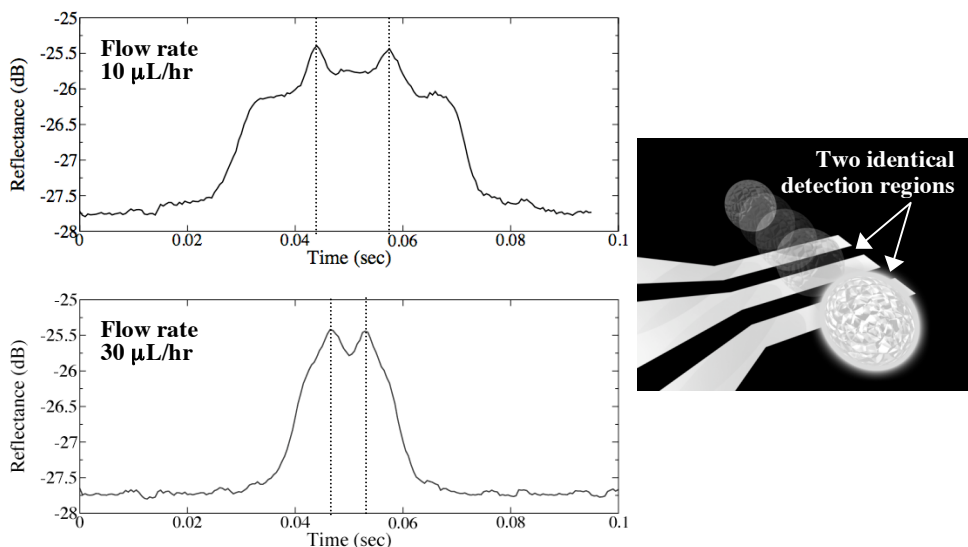


Figure 4: High-resolution transit-time measurements for 15 μm polystyrene beads. Two distinct peaks are observed as the bead crosses the twin detection regions between the signal and the adjacent ground electrodes (40 μm peak-to-peak distance).

In conclusion, we demonstrate a high-throughput microfabricated Coulter counter that operates above 240 MHz, has intrinsic detection rates reaching 10 MHz. The device was used to demonstrate the detection of human breast tumor cells in a $50 \times 50 \mu\text{m}^2$ microchannel.

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