ABSTRACT

We report the design and the electrical and microfluidic characterization of a device that improves signal strength and increases the detection levels in microfluidic impedance cytometry. Low-conductivity aqueous and two-phase dielectric sheathing allows the use of wider, more easily fabricated channels, increases the precision of cell- and particle-size measurements, and decreases the smallest detectable object size. For aqueous dielectric sheathing with a fixed excitation voltage of 400 mV_{rms}, the highest relative signal strength measured was 1.4 ± 0.6% for a 27-µm core. The use of a two-phase system and a core width of 33 µm resulted in a signal strength of 5.1 ± 0.5%. This two-phase system increases SNR by 23 dB compared to the aqueous system.

KEYWORDS: Impedance cytometry, Microfluidics, Dielectric focusing, Signal improvement

INTRODUCTION

Impedance cytometers are increasingly used within the biomedical community for counting and measuring the sizes and/or dielectric properties of particles and cells, and single and populational cell analysis is increasingly important for applications from drug discovery to diagnostics [1]. Microfluidic technologies have enabled the use of miniaturized Coulter counters and micro-impedance cytometers for label-free cell analysis [2, 3]. The use of microfluidics can improve performance relative to traditional cytometers by implementing a low-conductivity aqueous dielectric sheath [4-6], but a systematic approach to the design of a device utilizing this technique is lacking. Using two-phase flow can further improve performance by eliminating diffusion, a limiting factor in all-aqueous systems. It will also thus widen the range of flow rates that can be used and make smaller core widths possible. Use of oil as sheath fluid was recently demonstrated, but implications for detection were not reported [7].

EXPERIMENTAL

Our microfluidic impedance cytometer, Figures 1 and 2, uses a fluidic layer fabricated from 50-µm-thick, double-sided adhesive film (Adhesives Research, Glen Rock, PA) using a CO₂ laser engraving and cutting system (Universal Laser Systems, Scottsdale, AZ). Two sets of 20-µm-wide platinum electrodes separated by 100 µm, patterned on Borofloat® glass using standard photolithography and sputtering, were used for differential measurements. The fluidic layer was sandwiched between two glass chips and bonded using a hot plate at 70°C and a hand roller. Fluidic connections were fabricated from polydimethylsiloxane (PDMS) and bonded to the top glass chip with the aid of air plasma.

Figure 1: (A) Illustration of the components of the microfluidic impedance cytometer. A laser-cut, 50-µm-thick, polyester-supported adhesive layer is sandwiched between two glass chips with 20-µm-wide Pt microelectrodes separated by 100 µm. (B) Photograph of the actual device in an acrylic fixture providing fluidic and electric connections and a U.S. quarter dollar for scale. The pogo® pins used for electric connections are not attached to the fixture in this image.
Figure 2: The fluidics layout of the microfluidic impedance cytometer allows for hydrodynamic focusing of the sample, dielectrophoretic focusing/centering (5 MHz, 1.6 \(V_{\text{rms}}\)), and impedance detection of single cells/particles.

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The effects of dielectric focusing were characterized using both aqueous and two-phase systems. Deionized water (D.I.), \(\varepsilon_r \approx 80\), was used as the aqueous dielectric sheath whereas heavy mineral oil (EMD Chemicals Inc., Darmstadt, Germany), \(\varepsilon_r \approx 2.3\), was used for two-phase experiments. For both, a conductive core of phosphate-buffered saline (PBS) with 10-µm polystyrene particles (61946, Fluka Analytical) was hydrodynamically focused at various flow ratios to achieve and characterize varying core widths. Gravity flow was used for all aqueous experiments due to the high sensitivity of the system to small pressure fluctuations created by conventional syringe pumps. For the two-phase experiments, a syringe pump (EW-74900-20, Cole-Parmer, Vernon Hills, IL) was used due to the higher pressures needed to drive these flows. The particle velocities were kept at 6.3 mm/s for the aqueous experiments and at 43 mm/s for the two-phase experiments.

As varying particle positions within the channel are a common cause of signal variation in impedance cytometry [8], negative dielectrophoresis was applied to center the particles in the channel, homogenizing their velocities. A signal of 5 MHz and 1.6 \(V_{\text{rms}}\) was applied to focusing electrodes in the hydrodynamic focusing zone, producing a negative dielectrophoretic force centering the particles.

Figure 3 shows a block diagram of the detection system. A lock-in amplifier (SR830 DSP, Stanford Research Systems, Sunnyvale, CA) was used to detect particles passing the microelectrodes. A frequency of 65 kHz and a fixed excitation voltage of 400 mV\(_{\text{rms}}\), below the water hydrolysis window) was used for all measurements. The in-phase component was high-pass filtered with a 1 Hz cut-off filter (SR 650, Stanford Research Systems, Sunnyvale, CA) to remove any DC offset. The filtered signal was then acquired through a PCI-6024E DAQ-card (National Instruments, Austin, TX) and analyzed using MATLAB\(^\circledR\) (Mathworks, Natick, MA). As the base current, \(I_{\text{base}}\), passing through the channel is different for each core width, the signal strength (relative change in current for a particle passing the electrodes, \(\Delta I/I_{\text{base}}\)), as well as the signal-to-noise ratio (SNR, \(\Delta I/I_{\text{noise}}\)), were calculated for each core width. Core widths were measured from channel micrographs.

RESULTS AND DISCUSSION

Particles were analyzed for core widths ranging from 145 µm to 27 µm in the aqueous system and for a single core width of 33 µm for the two-phase system. Results (Figure 4) show the relative detected signal from the passage of a
single particle increasing with decreasing core width for aqueous sheaths, culminating at $1.4 \pm 0.6\%$ for the smallest (27 $\mu$m) core. For the two-phase flow, a signal of $5.1 \pm 0.4\%$ was measured for a 33 $\mu$m core. The SNR shows no significant increase with decreasing core size for the aqueous setup; however for the two-phase flow an improvement of 23 dB is measured.

Figure 4: (A) Average detection signal, $\Delta I/I_{\text{base}}$, for the passage of single 10-$\mu$m polystyrene beads in PBS with a range of core sizes (all aqueous) and for a two-phase flow (oil sheath, 33 $\mu$m core). For a fixed excitation voltage of 400 mV$_{\text{rms}}$, a detection signal of $1.4 \pm 0.6\%$ is measured for a 27-$\mu$m aqueous core, whereas the two-phase system has a signal of $5.1 \pm 0.5\%$. (B) The signal-to-noise ratio for the various core sizes, showing no significant dependence on core size for the aqueous cores. For the two-phase system, a 23 dB improvement compared to the smallest aqueous core is observed. Error bars correspond to $\pm 1$ standard deviation for averages of 21 measurements.

CONCLUSION
There are many advantages of using dielectric sheathing in a microfluidic impedance spectrometer. Hydrodynamic focusing enables the use of larger channels that are simpler to fabricate, less prone to clogging, and need lower pressure to drive the fluid. Utilizing a dielectric sheath flow improves both the detected signal strength and the SNR for single-particle measurements without increased excitation voltages and currents, enabling the analysis of particles and cells with smaller sizes or lower dielectric contrast. Using a two-phase system that is immune to diffusion effects at the interface between the core and sheath also results in decreased signal variability, which will translate to higher precision when performing size measurements and comparing the signal response to that of a known particle size. For the low frequency used in this study, the detected signal is dependent only on the size of the object and the microparticles used are expected to result in very similar signals to cells [9]. It’s anticipated that the use of higher frequencies will further improve the signal which should improve the size resolution and make this approach more suitable for cells.

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REFERENCES

CONTACT
M. Evander, Tel: +1-650-723 5646; evander@stanford.edu