Overcoming the sensitivity vs. throughput tradeoff in Coulter counters: A novel side counter design

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Microfabricated Coulter counters are attractive for point of care (POC) applications since they are label free and compact. However, these approaches inherently suffer from a trade off between sample throughput and sensitivity. The counter measures a change in impedance due to displaced fluid volume by passing cells, and thus the counter’s signal increases with the fraction of the sensing volume displaced. Reducing the size of the sensing region requires reductions in volumetric throughput in the absence of increased hydraulic pressure and sensor bandwidth. The risk of mechanical clog formation, rendering the counter inoperable, increases markedly with reductions in the size of the constriction aperture. We present here a microfluidic coplanar Coulter counter device design that overcomes the problem of constriction clogging while capable of operating in microfluidic channels filled entirely with highly conductive sample. The device utilizes microfabricated planar electrodes projecting into one side of the microfluidic channel and is easily integrated with upstream electronic, hydrodynamic, or other focusing units to produce efficient counting which could allow for dramatically increased volumetric and sample throughput. The design lends itself to simple, cost effective POC applications.

1. Introduction

Flow cytometry is a versatile, high throughput fluorescence-based approach for cell counting and sorting (Shapiro, 2004). However, current flow cytometers are expensive, complicated, and require trained personnel, making it unsuitable for point of care (POC) applications. One of the most promising alternative approaches is an impedance-based cytometry (i.e., a “Coulter counter”), which measures electrical impedance changes as cells flow through a sensing region (Coulter, 1953; Coulter, 1956). Counters using the Coulter principle have the advantage of a simple, cost effective, and label free electronic detection method that lends itself to POC applications.

Though several embodiments of microfabricated Coulter counters (Rajan et al., 2016; Watkins et al., 2011) and resistive pulse sensors (Peng and Li, 2018) geared toward POC applications have been reported, these designs contain technical trade-offs between sample throughput and counter sensitivity. Since Coulter counters measure the change in impedance resulting from displaced volume by passing cells, the fraction of displaced sensing volume is directly proportionally tied to the counter’s signal. When operating under limited hydraulic pressure and sensor bandwidths, obtaining smaller sensing regions requires decreases in volumetric throughput. As constriction apertures are reduced in the pursuit of smaller sensing regions the risk of mechanical clog formation increases significantly, rendering the counter inoperable (Zheng et al., 2013). To overcome these limitations, higher throughput multiple channel configurations have been explored (Zhe et al., 2007), but solutions to interchannel crosstalk in multiple channel designs (Jagtiani and Zhe, 2011) are not efficient and dramatically increase complexity. Existing solutions to mitigate clog formation, such as dynamic pneumatic valves (Kim and Kim, 2013), introduce significant complexity and circuitry.

Modern solutions utilize hydrodynamic focusing with nonconducting sheath solutions to avoid the risk of clog formation (Bernabini et al., 2011; Larsen et al., 1997; Nieuwenhuis et al., 2004). Planar
electrodes span the entirety of the fluidic channel width but the impedance between sensor electrodes is dictated by the intervening volume of conductive fluid. This approach mitigates clogging risk by retaining a wider fluidic aperture while imposing the requirements of a second nonconductive solution needed for device operation (Scott et al., 2008). We demonstrate a microchip device design that mitigates clogging risk with similar ease while eliminating the need for nonconductive sheath flows as seen previously in the literature. We limit the electrical sensing volume by confining the sensing electrodes to one edge of the microfluidic channel, permitting operation in a channel filled entirely with a conductive solution with minimal expected degradation of counter sensitivity. The design readily integrates with upstream means of laterally actuating particles of interest within the sample (i.e. dielectrophoresis (DEP), acoustic pressure) because of its simplicity. Here we use upstream hydrodynamic focusing simply to demonstrate the device operation.

2. Materials & methods

2.1. Device fabrication

The devices were fabricated in the Yale Cleanroom facility. Devices were fabricated on a silicon wafer with a 2 μm thermally grown silicon dioxide layer (Silicon Valley Microelectronics). Electrodes were patterned by standard photolithography, with a bilayer LORSA and Shipley S1805 resists for a liftoff mask. Wafers were metalized with 15 nm of titanium followed by 285 nm of gold. The resist was lifted off in hot NMP. After cleaning and coating with a protective resist, wafers were diced into individual chips.

Microfluidic channels were fabricated from a SU-8 photoresist mold patterned on a 4 inch silicon wafer. The channel height was determined by the SU-8 thickness (18.2 μm), and the designed channel width was 1 mm. The microfluidic channels were cast from the SU-8 mold with a 10:1 mixture of poly (dimethylsiloxane) (PDMS) base and curing agent (Dow, Dowsil 184 Silicone Elastomer Kit) and cured in an oven at 80 °C for 2 h. Input and output holes were punched into the PDMS layer (Electron Microscopy Sciences, Rapid Core 0.75 mm), and the channels were cleaned with isopropyl alcohol and deionized water. Channels were bonded to fabricated silicon chips by exposing both surfaces to oxygen plasma at 40 Watts for 20 s (Glow Research, AutoGlow), then immediately bringing the bonding surfaces into contact and baking on a hotplate at 90 °C for at least 10 min. Teflon tubing (Component Supply Co., STT-28-C) was inserted into the input and output holes in the PDMS and a syringe of sample and syringe needle (BD Biosciences, 305,110) connected to the input tubing.

2.2. Instrumentation and measurement procedure

The devices were interfaced electrically with a custom built sample mount and printed circuit board (PCB) that mated coaxial adapters to contact pads on the chips via a spring loaded header. An Agilent 33210A function generator provided a signal at 78 kHz to the Coulter counter structures (Kobos, 2019). The PCB contained an instrumentation amplifier which provided a differential gain of 15.9 to the bridge circuit signal. An SR830 lock-in amplifier measured this output voltage, which was digitized and recorded with a Tektronix DPO4104 oscilloscope and a MATLAB acquisition routine.

Sample solutions were prepared by mixing 990 μL of 1x phosphate-buffered saline (PBS) and 10 μL of polystyrene bead sample (Spherotech). The solution was then mixed for 30 s using a vortex mixer. A
second buffer solution of 1 mL of 1x PBS was also prepared. These were loaded into individual 1 mL syringes, and placed in two syringe pumps (New Era Pump Systems, Inc. NE-1000) and connected to the device inlets. The bead sample flow rate was set to 8 μL per minute, while the buffer solution pump was programmed to 48 μL per minute. Once a steady state flow regime was observed, the data collection was started.

2.3. Coulter counter operating principles

Our coplanar Coulter counters, as depicted in Fig. 1a and b, employ a three electrode impedance bridge similar to previous designs (Gawad et al., 2003; Watkins et al., 2011). The Coulter principle relies on a displaced volume of conductive solution between the electrode structures by an insulating particle (Coulter, 1956; Zhe et al., 2007), which generates a change in sensor impedance proportional to the said volume. For example, an insulating spherical particle of diameter \(d\) within a rectangular channel of length \(L\), width \(w\), and height \(h\) containing a fluid of resistivity \(\rho\) causes a change of resistance \(\Delta R\) (DeBlois and Bean, 1970) of:

\[
\Delta R = \rho \pi d^2 / \left[4(wh)^2\right]
\]  

(1)

in the limit where \(L \approx h\) or \(w\), and for \(d^2 \ll wh\). The magnitude of the sensor signal scales with the volume of the particle or cell relative to the overall sensor volume (Cheung et al., 2004; DeBlois and Bean, 1970).

In an impedance bridge approach, a sinusoidal excitation signal applied to the middle electrode drives current to flow through the solution to the outer sensing electrodes, which are connected to the circuit ground by resistors. The potential that forms at each sensing electrode is governed by the ratio of the bridge resistor to the solution impedance between the excitation and sensing electrodes. The solution resistance dominates the sensor impedance at our chosen operating frequency. We demodulate the differential signal between the two sense electrodes with a lock-in amplifier and record the time-domain output.

When a particle passes between the excitation and sensing electrodes, the solution resistance is temporarily increased, reducing the voltage measured at the sensing electrode. Fig. 1c illustrates the process by which a typical Coulter counter signal is generated in a three electrode geometry. As the particle approaches (1) and enters (2) the sensing region formed between the first and second electrodes, the solution resistance is increased due to the volume displaced by the particle. As the particle passes over the second electrode (3), the solution resistance returns to its normal operating state. The process repeats as the particle flows between (4) the second and third electrodes before finally exiting (5) the sensing region. The output of this configuration is a voltage signal proportional to the difference in resistance between the first and
second sensing regions. The relevant instrumental settings for Fig. 1c are given in the Methods section. In a previously published work, we have demonstrated the use of the constricted Coulter counter device design for counting and characterizing the size of T cell immune cells as an example of a clinically relevant POC application (Han et al., 2020).

The side counter chip features the same electrode and detection electronics of the conventional Coulter counter (Fig. 1a), however the electrodes are placed at the side of an unconfined channel (Fig. 1b). By focusing target particles to be counted into the 100 μm lateral extent of the electrode region, dramatic increases in sample throughput without channel clogging can be achieved (Fig. 2b) as compared to the constricted channel, which is readily subjected to clogging issues at high throughput rates (Fig. 2a). In these constriction devices (width = 20 μm, height = 17.1 μm, length = 300 μm), we observe clogging (with 6 μm beads flowing at 0.5 μL/min) after only 30 s.

In this proof of principle demonstration, we use hydrodynamic focusing with a bifurcated fluid input to emulate lateral target particle actuation upstream. Targets (beads) are introduced on the counter side of the channel, and a focusing buffer flows through the opposite side. No mixing occurs over the length scale of the counter due to the laminar flow conditions within the channel; we estimate the Reynolds number of the flow to be 1.85. This approach is extendable to any of a number of upstream dynamic focusing or sorting systems, such as a DEP lateral sorter (Cheung et al., 2004). The microchip is electrically interfaced with a custom PCB and mounted under a microscope objective for optical monitoring of bead passage events, as shown in Fig. 2c and schematically illustrated in Fig. 2d.

3. Results and discussion

While the increased overall channel volume of the side counter design would suggest that the detected signal would be reduced, the electric field from the electrodes decays quickly with distance, thereby containing the sensing volume to a region predominantly around the electrode structure as shown in Fig. 3a and Supplementary Fig. S1. The sensing region of the conventional Coulter counter is mechanically constrained by the microfluidic channel; we expect the sensing region of the side counter Coulter counter to be electrically constrained by the fringing electric field.

To substantiate this hypothesized electrically constrained sensing volume, we perform simulations with COMSOL Multiphysics 5.5 (COMSOL Inc., Burlington, MA, USA) to determine the impedance for a particle at different lateral positions across the channel as shown in Fig. 3. The simulation shows that the impedance change is constant within the electrode region and falls off at the edge of the electrode protrusion into the channel (Fig. 3b), confirming the electrical confinement effect. The simulation data is normalized to the value obtained for a constricted counter geometry without any edge effects (with channel walls at y = 0 and y = 100 μm). The length of the electrodes exposed to the solution is the same in both cases, but the fringing fields sample additional liquid volume, slightly increasing the effective sensing volume. As a result, the relative impedance change and thus signal magnitude decreases by ~20% for a fixed target diameter.

To characterize the behavior and performance of the side counter, polystyrene beads with nominal diameters of 6.08 μm or 8.87 μm were flowed through the device’s sample input channel at 8 μL/min and buffer solution introduced in the second input at 48 μL/min for hydrodynamic focusing. The device channel is 1 mm in lateral width and 18.2 μm in height, and each of the three electrodes is 20 μm wide along the flow direction with 20 μm interelectrode spacing. The electrodes protrude approximately 115 μm into the channel.

As the beads pass over the electrodes, they produce the characteristic Coulter counter signal trace, as shown in Fig. 4a for two different bead sizes. As expected, the signal scales with particle size. To determine the ability of the counter to calibrate particle dimension, counter traces were taken over time to obtain a histogram of signal amplitudes. Fig. 4b shows a representative 1 s duration signal trace produced by the 6.08 μm diameter beads showing multiple signals of varying amplitude. Particle passage event signals were automatically extracted by setting a threshold parameter determined by the signal to noise ratio, then algorithmically fit to extract an accurate peak amplitude and transit time (Cheung et al., 2004). A histogram of the cumulative counts from 1 min of signal acquisition with the 6.08 μm beads is shown in Fig. 4c, normalized to the maximum bin count and fit with a Lorentzian. These signals are plotted as the cube root of the normalized signal amplitude since this would then be linear with bead diameter (Eq. (1) and Cheung et al., 2004). Supplemental Fig. S2 shows a representative calibration scale with size. The histogram has been normalized to the maximum bin count and fits with a Lorentzian, with a peak amplitude of 0.023 corresponding to a 4.02 mV signal amplitude, representative of the trace of Fig. 4a. The distribution in signal amplitude is attributable to both the particle size dispersion (0.503 μm dispersion for 6.08 μm beads) as well as vertical position in the channel.

In addition to particle size information, the signals also encode information about particle velocity, which has a dependence upon vertical particle position. From Fig. 4a, we have a transit time of approximately...
Fig. 4. a) Representative single bead transit signals produced by 8.87 μm (orange) and 6.08 μm (blue) diameter polystyrene beads. b) A 1 s duration signal trace produced by 6.08 μm diameter polystyrene beads showing multiple passage events. c) A histogram of the cube root of relative signal magnitudes produced by 6.08 μm diameter polystyrene beads for approximately 4000 counts. The fit Lorentzian peak is at 0.0233 (with a FWHM of 0.00463), which corresponds to a 4.02 mV signal amplitude. d) A plot of normalized signal amplitude (converted to a quantity proportional to bead diameter) versus transit time, illustrating the effect of vertical bead distribution. As the beads drop within the channel, their velocities decrease, leading to a greater transit time. When coupled with their closer proximity to the sensing electrodes, the larger beads’ slower transit times directly translate to stronger signal amplitudes, which accounts for the tailing behavior. e) Simulation model of the system, with varying bead positions along the channel as indicated by the dashed outlines. In the figure shown, the particle (purple) currently is at coordinates x = 60 μm. For all x positions, the bead height above the channel floor is z = 10 μm. Note that the particle size is to scale. f) Simulation results of the relative differential impedance for 6.08 μm and 8.87 μm beads (plotted in red and blue, respectively) as they pass over the counter.
0.5 ms from signal maximum to minimum (corresponding to x = 50 μm–90 μm). The two dimensional histogram of signal amplitudes versus transit times shown in Fig. 4d reveals a strong correlation between the signal amplitude and bead transit times, with a mean transit time of 0.57 ms, corresponding to a velocity of 0.07 m/s. This agrees very well with a model of the particle velocity in the center of the stream to be 0.07 m/s (shown in Supplementary Fig. S3c, assuming a parabolic velocity profile typical of laminar flow). Fig. 4d also shows how the bead transit times decrease, the detection amplitude tends to converge to a medium value, indicating that the faster traveling beads tend to be located near the vertical center of the channel as expected. Representative examples of slower transit time, higher signal amplitude traces are shown in Supplementary Figs. S3a and S3b.

These experimental signal amplitude and transit time values agree qualitatively well with impedance simulations (COMSOL), as shown in Fig. 4 e-f. The impedance measured between each electrode pair was modeled for the two different bead sizes used at different particle positions along the length of the channel x (at fixed height z = 10 μm), as shown schematically in Fig. 4e. Fig. 4f shows the calculated signal from the impedance difference for a detected bead, normalized to a maximum impedance difference of 137 Ohm at x = 50 μm. Direct comparison of the simulation and experiment by using the calculated impedance is not possible, due to the impedance of the electric double-layer at the sensor-solution interface, parasitics both on-chip and on the measurement PCB, and the integration time constant of the lock-in amplifier. However, ratios will eliminate these unknowns. The ratios of calculated impedance results and experimentally obtained signal amplitudes agree well: 1 versus 0.37 (137 Ohm vs. 51 Ohm) for the simulations and 10.32 mV versus 4.02 mV from the experimental results, with ratios of 2.7 and 2.57, respectively. As expected and consistent with the experimental results, a larger particle diameter results in a larger detection signal due to the more significant change in impedance when passing between the electrodes. The detected signal magnitude implies a system detection sensitivity of approximately 75 μV/Ohm.

4. Conclusions

Flow cytometry is a critical technique for cell counting and sorting, and performs tests such as complete blood counts (CBCs). However, existing systems are not portable. One of the most promising alternative approaches for portable POC flow cytometry is an impedance based Coulter counter, which has the advantage of being simple, cost effective, and a label free electronic detection method that both counts and resolves particle size. However, the ability to perform portable and rapid diagnostics with lab on a chip cell counters have been stymied due to low throughput and clogging issues. Past researchers have employed hydrodynamic focusing with nonconductive sheath flows to address clogging in microfluidic Coulter counters. Employing secondary, nonconductive solution to focus the conductive sample over a narrow region of the microfabricated electrodes still constrain the sample of interest to flow through a narrow fraction of the microfluidic channel. Correspondingly higher drive pressures are needed to achieve desired sample velocities over the counter.

The side counter approach presented here simultaneously remedies both throughput and clogging concerns with a simple electronic detection method that remains capable of both counting and resolving particle size. By placing the sensing electrodes along one side of the solution channel, an electrical sensing region is developed as opposed to typically mechanically constrained sensing regions.

The side counter approach mitigates clogging skin to sheath flow implementations. However, the side counter obviates the need for a second fluidic inlet, and corresponding buffer reservoir for POC applications. Provided an upstream actuator (DEP, piezoacoustic SAW) for particles of interest, the sample may flow through the entirety of the microfluidic channel.

Investigating the impact of our sensor electrode placement, an analysis of the experimental signal amplitudes and transit times agree well with simulation results, further reinforcing the device’s capabilities counting and sizing micron scale particles. Through integration of microfluidic actuators with a unique Coulter counter device architecture, high throughput and reliability are ensured while not compromising in the realms of portability, design simplicity and economic feasibility. As such, this detection technique and facilitating device architecture have many direct applications to POC field cases, with the ready capacity to scale for portable applications.

CRediT authorship contribution statement

Daniel T. Bachschitz: Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization.
William Polsky: Validation, Formal analysis, Investigation, Writing - original draft, Visualization.
Zachary Kobos: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing - original draft, Visualization.
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Lukas Menze: Software, Formal analysis, Writing - original draft, Visualization.
Jie Chen: Software, Writing - original draft, Writing - review & editing, Visualization, Supervision, Funding acquisition.
Mark A. Reed: Methodology, Resources, Data curation, Writing - original draft, Writing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bios.2020.112507.

Data and materials availability

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data or codes related to this paper may be requested from the corresponding author.

References

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**Supplemental information**

**Supplemental Figure S1.** Simulations of electric field lines in the side counter system illustrate the region of effective particle detection, as shown by the electric field line density attenuation that occurs near the end of the electrode projection into the channel. a) When a particle is located inside the electrode region, the response resembles that of a constricted counter, underscoring the effectiveness of the side counter. b) As the particle location is moved farther out into the channel, beyond the electrode, the side counter’s voltage response decays.
Supplemental Figure S2. Counter response versus particle size. A mixture of spherical particles of different sizes, with nominal sizes of 4.45 μm, 6.08 μm, and 8.87 μm diameter in PBS. A histogram of the voltage ratio response ΔV/V₀ is determined, and the cube root of this maximum (error bars FWHM) is plotted versus the optically determined particle diameter. The response linearly correlates with sample diameters (from Patrick et al., 2020).
Supplemental Figure S3. Side counter single-bead voltage signals produced by a) 8.87µm and b) 6.08 µm diameter polystyrene beads. Their significantly larger amplitudes and larger transit times are attributed to their positions within the channel. c) Calculated estimate of particle velocity distribution expected within the channel along the height-width cross-section of the side counter’s channel, assuming laminar flow in a rectangular channel (Purday, 1949) and then including approximated particle retardation along channel walls (Goldman et al., 1967). As a result of flow edge effects, beads that find themselves lower in the channel travel at lower flow rates, translating into slower transit times. Due to their proximity to the electrodes, a larger signal amplitude is observed.