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## Highly selective biomechanical separation of cancer cells from leukocytes using microfluidic ratchets and hydrodynamic concentrator

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The separation of cells based on their biomechanical properties, such as size and deformability, is important in applications such as the identification of circulating tumor cells, where morphological differences can be used to distinguish target cancer cells from contaminant leukocytes. Existing filtration-based separation processes are limited in their selectivity and their ability to extract the separated cells because of clogging in the filter microstructures. We present a cell separation device consisting of a hydrodynamic concentrator and a microfluidic ratchet mechanism operating in tandem. The hydrodynamic concentrator removes the majority of the fluid and a fraction of leukocytes based on size, while the microfluidic ratchet mechanism separates cancer cells from leukocytes based on a combination of size and deformability. The irreversible ratcheting process enables highly selective separation and robust extraction of separated cells. Using cancer cells spiked into leukocyte suspensions, the complete system demonstrated a yield of 97%, while enriching the concentration of target cancer cells 3000 fold relative to the concentration of leukocytes. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4812688>]

### I. INTRODUCTION

The separation of cells based on their biomechanical properties, such as size and deformability, has some key advantages over separation methods based on affinity capture because of the ability to avoid using cell surface markers, as well as the potential to extract viable cells after separation. An area of particular interest for this type of cell separation is in the case where target cells are relatively rare compared to the background cell population. Applications for rare cell capture range from stem cell research<sup>1</sup> to cancer diagnostics.<sup>2,3</sup> When the total number of target cells in a sample is small, extremely selective technologies must be employed to avoid missing any of these potentially valuable cells. Affinity capture methods can be unreliable when the cell surface markers used are absent on a portion of the target cells as is the case for epithelial markers used to detect circulating tumor cells (CTCs) in the peripheral blood of cancer patients.<sup>4</sup> Biomechanical separation processes offer an alternative to affinity capture that may allow the study of previously uncaptured cells.

Advances in microfluidic technologies have facilitated the development of novel approaches to biomechanical cell separation. Not only do these technologies enable the fabrication of structures at the length scale of individual cells, they also allow precise control over the flow of minute volumes of liquid. Current microfluidic separation techniques can be grouped into hydrodynamic methods,<sup>5–16</sup> dielectrophoresis,<sup>17–20</sup> and filtration methods.<sup>21–30</sup> Hydrodynamic methods use micro-scale geometries to alter the flow of particles based on differences in size. This approach typically has greater throughput than filtration, but has limited selectivity.<sup>7,8,13</sup> Dielectrophoresis can

achieve high yield but suffers from low throughput, and enrichment is typically lower than filtration.<sup>17,19</sup>

Filtration methods involve flowing a sample through an array of micro-scale constrictions to separate cells based on a combination of size and deformability. This approach is more selective than hydrodynamic methods but is limited by clogging. Specifically, as the filter constrictions become progressively blocked by captured cells, the overall hydrodynamic resistance of the filter changes unpredictably, altering the pressure difference experienced by cells being deformed through the remaining open pores.<sup>30,31</sup> Additionally, the persistent force on the trapped cells can cause cytoskeleton remodeling and increased cytoadhesion, which further degrades filter performance.<sup>30,32</sup> Finally, in many filtration techniques, captured cells cannot be extracted from the filter microstructure, limiting downstream processing options such as molecular characterization, genomic analysis, and propagation in culture or xenograft models.<sup>33,34</sup>

We previously reported a microfluidic ratchet mechanism that uses irreversible transport of cells through funnel shaped constrictions to sort cells based on a combination of size and deformability, while avoiding clogging by periodically clearing the filter microstructure.<sup>35</sup> We demonstrated the ability to separate two equally represented cell types in a dilute mixture using this ratchet mechanism with an enrichment of approximately 100 fold. In this paper, we leverage the ratchet mechanism to create an automated microfluidic platform that enables both the separation and extraction of rare cancer cells from background leukocytes based on size and deformability. This platform has two components. The first component is a size-based hydrodynamic sample preparation stage that concentrates the sample by removing a large portion of the suspension fluid as well as some of the contaminant leukocytes. The second component is a cell separation stage involving unidirectional transport of cells through a 2D array of funnel constrictions using oscillatory flow. We characterize and optimize the performance of the concentrator and sorting device individually and then demonstrate highly selective separation of cancer cells from leukocytes using the combined system.

## II. MATERIALS AND METHODS

### A. Fabrication of silicon masters

Microfluidic devices are fabricated using PDMS and contain microstructures for a flow layer and a control layer assembled using multi-layer soft lithography. Inverse masters of the flow layer and control layer microstructures are fabricated on silicon wafers using photolithography. Patterns for the microstructures are drawn using DraftSight (Dassault Systems, Vélizy-Villacoublay, France) are then translated onto commercially produced optical photomasks. The microstructures for the flow layer silicon wafer are deposited onto a silicon wafer using three photolithographic layers. In the first layer, the silicon wafer is coated with SU-8 negative photoresist (Microchem, Newton, MA) and spun at a speed of 3000 rpm for 30 s. This wafer is baked on a 95 °C hotplate for 5 min followed by UV exposure through a high-resolution optical photomask (1 μm feature accuracy, Advance Reproductions, Andover, MA). The wafer is then baked at 65, 95, then 65 °C for 1, 4, and 1 min, respectively. The patterned wafer is developed using SU-8 developer (Microchem, Newton, MA) and washed with isopropanol. A second, low-resolution photomask (5 μm feature accuracy, CAD/Art Services, Brandon, OR) is aligned to the first pattern and used to define a second set of SU-8 features following the same procedure. To harden the patterned SU-8 microstructures, the wafer is placed on a hot plate and the temperature is ramped from 40 °C to 165 °C, increasing 15 °C every 10 min. The wafer is baked for 30 min at 165 °C. Finally, the hot plate temperature is ramped down at a rate of 50 °C every 10 min until 65 °C. A layer of SPR220-7.0 photoresist (Microchem, Newton, MA) is then spun onto the wafer at 600 rpm for 50 s. The edge bead is removed from the wafer manually with a clean wipe and the wafer is baked for 1, 5, and 1 min at 65, 95, and 65 °C, respectively. A third, low-resolution photomask (CAD/Art Services, Brandon, OR) is aligned to the previous set of patterns. The wafer is exposed and developed using MF319 developer (Microchem, Newton, MA). Finally, the wafer is baked on a hotplate at 95 °C for 5 min to allow the SPR220-7.0 photoresist layer to reflow. The final thickness of all three layer of photoresist is

approximately 25  $\mu\text{m}$ . The control wafer is fabricated with a single layer of SU-8 using a separate patterned mask following the same SU-8 fabrication procedures.

## B. Fabrication of PDMS devices

PDMS devices are fabricated using standard multi-layer soft lithography techniques.<sup>36</sup> The flow layer is formed using a 5:1 base to hardener ratio of Sylgard 184 silicone (Dow Corning, Midland, MI), and the control layer is formed using a 20:1 ratio. The flow and control layers are bonded together by diffusion in 65 °C oven for 2.5 h. The bonded flow and control layers are subsequently attached to a glass slide following 90 s activation in air plasma (Harrick Plasma, Ithaca, NY). Inlets, outlets, and reservoirs are punched manually using 0.5, 3.5, and 5 mm punches (Harris, Redding, CA).

## C. Sample preparation

Experiments were conducted using leukocytes and UM-UC13 (UC13) bladder cancer cells. Whole blood was drawn from healthy donors with informed consent into 6 ml EDTA blood collection tubes. Gradient density centrifugation was performed to isolate nucleated cells, which includes the leukocytes in the buffy coat.<sup>37,38</sup> In a 15 ml centrifuge tube, 2 ml of whole blood was carefully layered over 2 ml of Histopaque 1119 (Sigma-Aldrich, St. Louis, MO). The tube was centrifuged at 400 g for 40 min. The leukocyte layer was then transferred into a 15 ml tube containing 10 ml of HBSS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Invitrogen, Grand Island, NY) and centrifuged at 200 g for 10 min. The supernatant was removed and the cells were washed again with HBSS.

The UC13 cells were kindly provided by the Pathology Core of the Bladder Cancer SPORE at MD Anderson Cancer Center. They were cultured in MEM solution with the addition of 10% (v/v) fetal bovine serum, 1% L-glutamine, 1% MEM Non-Essential Amino Acids, 1% Sodium Pyruvate (Invitrogen), and 1% Penicillin Streptomycin (Fisher Thermo Scientific, Waltham, MA), and incubated at 37 °C in a humidified environment with 5%  $\text{CO}_2$ .

## D. Experimental setup and procedure

Fluid handling for the experiment was performed using a commercial pressure controller (Fluigent, Paris, France), and the custom made pressure board and software described previously.<sup>39</sup> Microscopy and cell counting were performed using an inverted microscope (TS-100, Nikon, Tokyo, Japan), and a CCD camera (DS-2MBW, Nikon, Tokyo, Japan).

Sample cells were suspended in MEM culture media with 15% Ficoll PM400, 5% bovine serum albumin (BSA, Invitrogen), and 0.2% Pluronic F-127 (Invitrogen). The Ficoll additive prevents cells from settling to the bottom of reservoirs due to gravity. BSA and Pluronic are blockers that prevent cells from non-specifically adsorbing to PDMS surfaces in the microfluidic device. Prior to each experiment, the microfluidic device was incubated for 15 min to allow the blockers to first adhere to the PDMS surfaces.

In preparing for each experiment, UC13 cells were stained using calcein AM (Invitrogen), while leukocytes were stained using Hoechst 33342 (Invitrogen). The initial count for the number of UC13 and leukocytes was determined manually using a hemocytometer after which UC13 cells were serially diluted before being combined with leukocytes at each experiment's target ratio. After separation, isotonic phosphate buffered saline was added to the collection reservoirs to dilute the Ficoll concentration to allow cells to settle at a single focal plane at the bottom of the reservoir. A microscope with encoded stage is used to take fluorescence images of the collection reservoir with a 4 $\times$  objective. The resulting images are stitched together using Microsoft Image Composite Editor (supplementary material, figure S1).<sup>40</sup> The number of target and contaminant cells is then counted manually from the composite images. The performance metrics of yield, purity, and enrichment is calculated from the count values. The uncertainty in these evaluations derives from the uncertainty associated with the hemocytometer and manual cell count. The uncertainty associated with cell count obtained by hemocytometer is considered

to be 15%.<sup>41</sup> The uncertainty associated with manual cell count is estimated in each experiment and arises from variable fluorescence levels due to differences in cell morphology, cytochemistry, or buoyant mass.

### III. DEVICE DESIGN

#### A. Overview

##### 1. System design

The cell separation device is composed of a hydrodynamic sample concentrator and a ratchet cell sorter integrated onto a single chip. The two components are isolated from one another using membrane microvalves. The sample is first processed using the concentrator to remove >75% of the fluid. This concentrated sample accumulates in an intermediate reservoir and is subsequently sent through the ratchet cell sorter for separation. Finally, the target cells are collected in a reservoir suitable for image analysis (Fig. 1).

##### 2. Performance metrics

The performance of cell separation devices can be evaluated using (1) the capture yield ( $\eta$ ), which is the percentage of target cells retained by the device; (2) the enrichment ratio (ER), which is the ability of the device to amplify the concentration of target cells relative to contaminant cells; and (3) purity, which is measured as the fraction of target to contaminant cells in the final output. Specifically for our experiments, these metrics are defined as

$$\eta = \frac{UC13_{final}}{UC13_{initial}} * 100\%, \quad (1)$$

$$ER = \frac{UC13/WBC_{final}}{UC13/WBC_{initial}}, \quad (2)$$

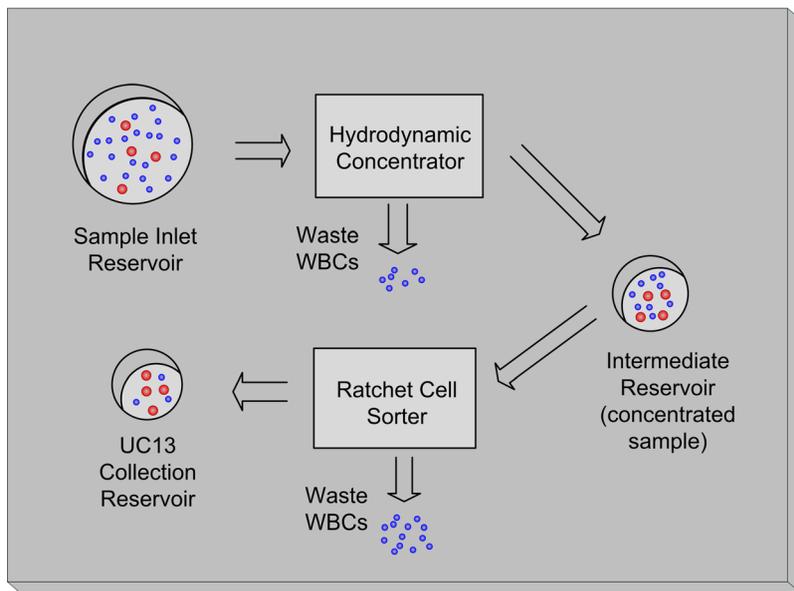


FIG. 1. Schematic of the cell separation chip. The cell mixture is first processed using the hydrodynamic concentrator to concentrate the sample and remove a portion of the contaminant leukocytes. The output accumulates in an intermediate reservoir. Next, the concentrated sample is separated using the ratchet cell sorter and the separated cancer cells are collected at the outlet.

$$\text{Purity} = \left( \frac{\text{UC13}}{\text{UC13} + \text{WBC}} \right)_{\text{final}} * 100\%. \quad (3)$$

In our experiments, we characterized this device using a cell mixture containing UC13 bladder cancer cells as target cells and leukocytes as contaminants. We measured UC13 cells to have a mean diameter of  $15.4 \pm 1.2 \mu\text{m}$  and leukocytes to have a mean diameter of  $10.3 \pm 1.8 \mu\text{m}$ . UC13 cells were selected for our experiments because these cells have a size distribution that overlaps with leukocytes but are relatively less deformable than leukocytes. In a previous study, we found that similar strains of cultured human cancer cells had a cortical tension of approximately 3 times that of typical leukocytes.<sup>42</sup>

## B. Concentrator design

The cell concentrator is an on-chip sample preparation component that removes the majority of the suspension fluid from the sample, as well as some of the contaminant leukocytes, to present a highly concentrated sample to the ratchet sorter. This component utilizes a pinch-flow design, previously used to hydrodynamically separate particles based on size.<sup>5,43,44</sup> The concentrator, as shown in Fig. 2, consists of a central channel that first constricts to an  $18 \mu\text{m}$  pinch and then expands into two equal width channels. Each of these channels has a port connected at its outside edge after the bifurcation point that leads to a waste outlet for fluid and contaminant leukocytes. The principle of this mechanism can be described as follows. Cells from the sample can be located at any lateral position in the central channel. At the pinch region, larger cells with diameters comparable to the pinch gap width are shifted to the streamline along the axial center of the channel by the constraint imposed by the pinch. This shifting is due to the size of the cells relative to the pinch gap. Large cells will completely fill the gap or be slightly compressed as they pass through, which centers them inside the channel. After passing through

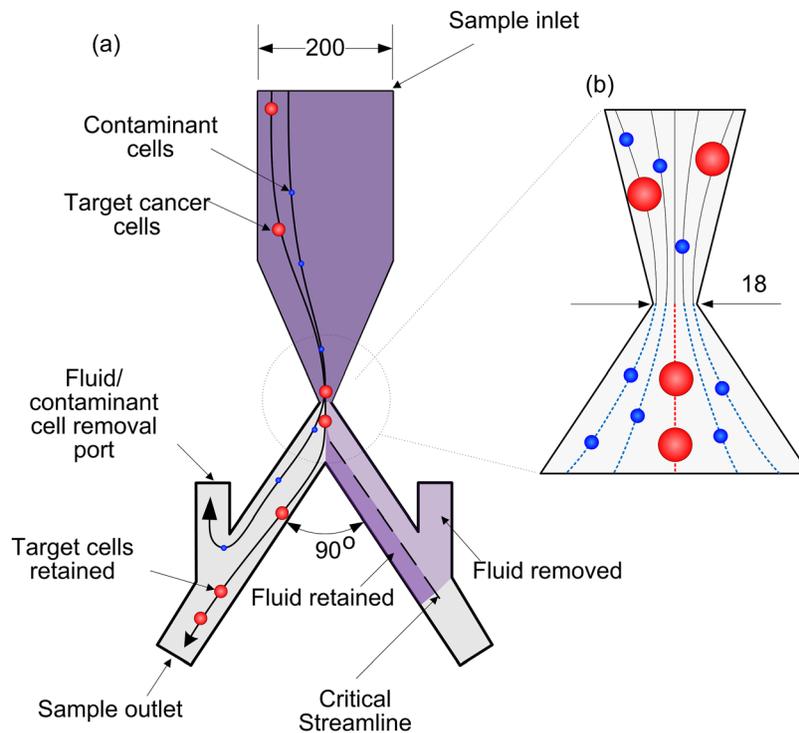


FIG. 2. (a) Schematic of the concentrator mechanism. The fluid removal ports remove fluid containing some contaminant cells while target cells are retained and carried downstream along the bifurcated channel. (b) Target cancer cells enter the pinch constriction from different streamlines but are re-aligned at the constriction to streamlines adjacent to the inner side of the bifurcation channels. Length dimensions are shown in microns.

the pinch, these larger cells continue to follow the central streamline to the inner wall of the bifurcated channels. On the other hand, smaller cells do not experience this alignment and continue to travel along the same streamlines as prior to the pinch (Fig. 2(b)). A portion of these smaller cells, along with a fraction of the suspending fluid, are removed by the side ports on the outside edge of the bifurcated channels. The amount of fluid removed is delineated by a critical streamline dividing the fluid into an inner fraction that is retained and an outer fraction that is removed (Fig. 2(a)). After fluid and cell removal, the collection channels converge downstream to carry the reduced sample on to a subsequent concentrator mechanism. The complete concentrator consists of four identical concentration mechanisms in series.

Using the density and viscosity of water, the average Reynolds number in the concentrator was estimated to be less than 0.001. In actuality,  $Re$  is even smaller since the viscosity of the liquid increases substantially with the addition of Ficoll. Under these conditions, the amount of fluid removed by the concentrator can be calculated analytically using an electrical circuit analogy to model the pressure and flow relationship inside the device. Specifically, the amount removed at the  $i$ th concentrator mechanism ( $i = 1, 2, 3, 4$ ) is a function of the flow resistances and pressure drops across the main channel and fluid removal channels as defined by

$$\frac{Q_i}{Q_{R,i}} = \left( \frac{P_i - P_{i+1}}{P_i} \right) \frac{R_R}{R_i}, \quad (4)$$

where  $Q_i$  is the combined flow rate at the output bifurcation channels;  $Q_{R,i}$  is the total flow rate at the fluid removal channels;  $P_i$  and  $P_{i+1}$  are the pressures at the inlet and outlets; and  $R_i$  and  $R_R$  are the hydrodynamic resistances of the bifurcation and fluid removal channels, respectively (Fig. 3).

Maintaining a constant flow ratio of  $Q_i/Q_{R,i}$  for each concentrator in the series ensures consistent retention of target cells across the entire system. A flow ratio in the range of 6–8 (Table 1) was experimentally verified to retain nearly 100% of the cancer cells, which corresponds to an  $R_R$  value approximately 50 times greater than  $R_i$ . At these flow ratios, the amount of predicted fluid removal ranges from 21.2% to 9.9% of the total volume from the first to last concentrator mechanism, respectively. The total amount of fluid removed is progressively less at each concentrator because the fluid volume is diminished after each, reducing the absolute amount of fluid the subsequent concentrator can remove under the same flow ratio conditions. This model predicts that the entire system is capable of removing 61.1% of the fluid passing through the device. These results were confirmed using computational fluid dynamics simulations (COMSOL MULTIPHYSICS 4.2). The cell suspension was modeled as a homogeneous Newtonian fluid. The simulation predicts a total fluid removal of 69.1%, which is comparable to the results obtained from the analytical study.

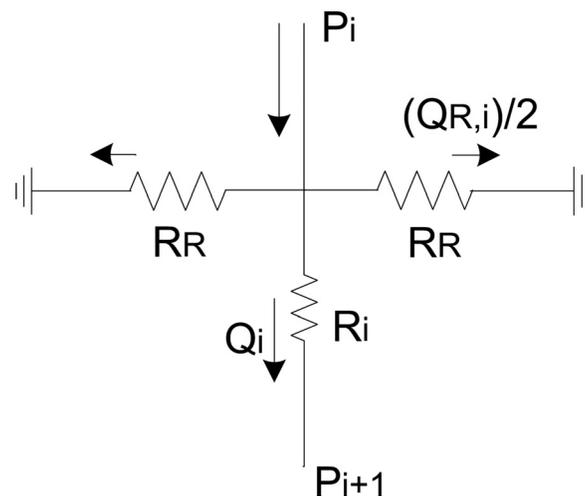


FIG. 3. Electric circuit analogy for a single concentrator mechanism. The outlet bifurcation channels are shown as a single combined resistance  $R_i$ .

TABLE I. Flow ratios and overall fluid removal performance of concentrator system.

Concentrator index (i)	Flow rate ratio $Q_i/Q_{R,i}$	% Removal calculated
1	7.4	21.2
2	6.7	18.2
3	8.2	11.8
4	7.8	9.9
Total	—	61.1

### C. Ratchet cell sorter design and characterization

The ratchet cell sorter uses a 2D array of microscale funnel constrictions that form microfluidic ratchet mechanisms to enable unidirectional transport of cells under oscillatory flow.<sup>45</sup> Supporting fluidic elements for this microstructure include inlet microchannels, target cell, and waste outlets and additional microchannel networks for flow control (Fig. 4(a)). Mixed cell samples are infused from the inlet microchannel, and the separated cells are purged from the sorting region using a cross flow from the buffer inlet. The flow rate of the infusion and purging processes are controlled using elongated microchannels at the inlet and outlet to provide a dominant hydrodynamic resistance to dictate the flow rate for a given applied pressure. The sorting region consists of a 2D array of ratcheting funnel microstructures arranged in 32 successive rows, with 128 funnels in each row. The funnels in successive rows are aligned with each other to generate a linear streamline from the bottom to the top of the sorting region. The funnel array has a gap opening of  $18\ \mu\text{m}$  at the bottom row, and this size decreases with successive rows to  $2\ \mu\text{m}$  at the highest row. The cut-off funnel size for the separation of UC13 cancer cells and leukocytes was determined experimentally to be  $6\ \mu\text{m}$ . Cells that cannot transit beyond the cut-off are directed to an on-chip collection reservoir, while cells that are able to move beyond the cut-off are directed into a waste reservoir (Figs. 4(c) and 4(d)). In order to sort cells effectively, the cells must be deformed through each funnel constriction with approximately the same force. To ensure an approximately equal hydrodynamic force, oscillation flow through the funnel array is distributed using a tree microchannel network, which sequentially bifurcates the single entrance stream into 128 equal streams.

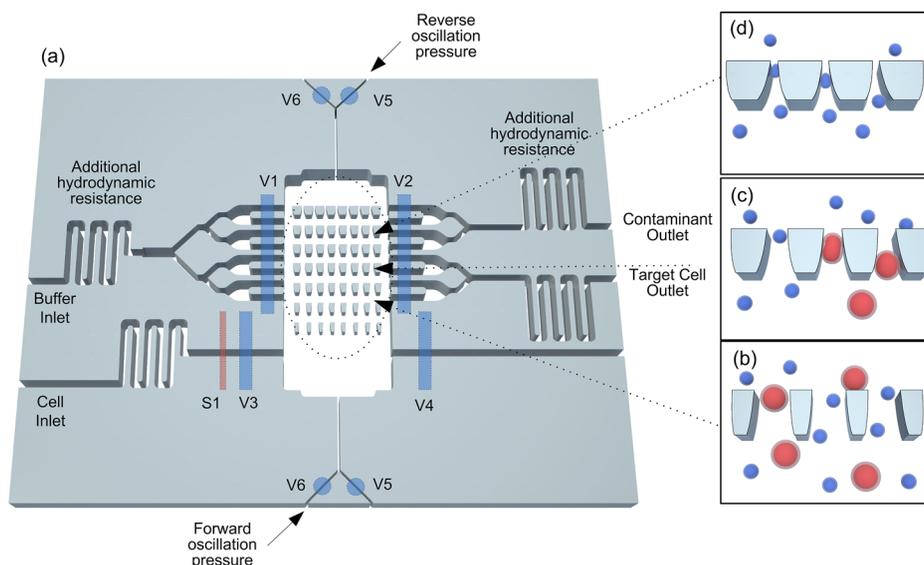


FIG. 4. (a) Schematic of the ratchet cell sorter. (b) A mixture of leukocytes (blue cells) and UC13 cells (red cells) enters the bottom row of the sorting region. (c) UC13 cells get trapped at the lower funnel rows before the critical  $6\ \mu\text{m}$  cut-off. (d) Leukocytes travel past the cut-off, ratcheting even further, and are eventually extracted into a separate outlet.

Sample cells are introduced into the sorting region in batches with a process controlled by membrane microvalves.<sup>36</sup> Initially, a plug of cells are infused into the bottom row of the sorting region using a horizontal flow created by opening valves V1 to V4 with V5 and V6 closed. Near the end of the infusion process, the sieve valve S1<sup>46,47</sup> is closed to create a cell-free section prior to closing V3 to stop the inletting completely. Next, the cell separation process begins by closing valves V1 to V4, and then introducing an oscillatory vertical flow using a fluidic H-bridge created using V5 and V6. Specifically, an upward flow can be generated using V5 closed and V6 open, while a downward flow is generated with V5 open and V6 closed. The upward and downward flow rates are controlled using the pressure applied at the forward and reverse oscillation ports, respectively. Finally, the separated cells are extracted using a horizontal flow introduced with valves V1 and V2 open to flush the cells into the collection outlets. This entire process is automated using a Visual Basic script from a control PC.

#### IV. RESULTS AND DISCUSSION

##### A. Concentrator characterization

The performance of the cell concentrator was verified experimentally using a mixture of leukocytes and UC13 cells at a ratio of 10:1. Specifically, this mechanism was found to retain >98% of the cancer cells and remove >75% of the fluid along with ~24% of the contaminant leukocytes. The percentage fluid removal measured is slightly greater than the modeling prediction likely due to errors in the estimate of hydrodynamic resistance of the microchannels.

##### B. Ratchet cell sorter characterization

Initially, the sample is infused into the device and sorted using an oscillatory flow with a velocity amplitude of  $\sim 200 \mu\text{m s}^{-1}$ . The oscillatory flow is biased towards the direction of the taper, where flow along the direction of the taper is applied for 4 s and against the direction of taper for 1 s. After 7 cycles of oscillation, the sample is completely separated with leukocytes transported to the top of the funnel array (Figs. 5(a)–5(c)) and UC13 cells retained in the lower rows (Figs. 5(d)–5(f), supplementary material, video S1).<sup>40</sup> Following separation, the cells are purged from the device into their respective collection reservoirs and then counted manually using an optical microscope at the end of each experiment.

We evaluated the ratchet sorter's performance using cell samples with UC13 cells spiked into leukocytes at ratios of 1:10, 1:100, and 1:600. As shown in Fig. 6, the capture yield is consistent across these experiments, with an average of 96%. Enrichment is also relatively

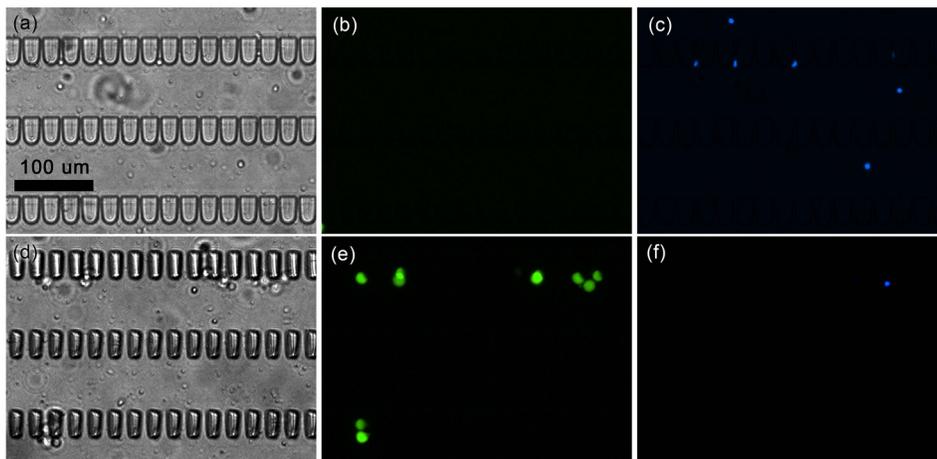


FIG. 5. After a sorting cycle, smaller, more deformable leukocytes stained in blue travel to the higher funnel rows shown in (a) brightfield, (b) green fluorescence, and (c) blue fluorescence. Larger and more rigid UC13 cancer cells are retained near the bottom, shown in (d) brightfield, (e) green, and (f) blue.

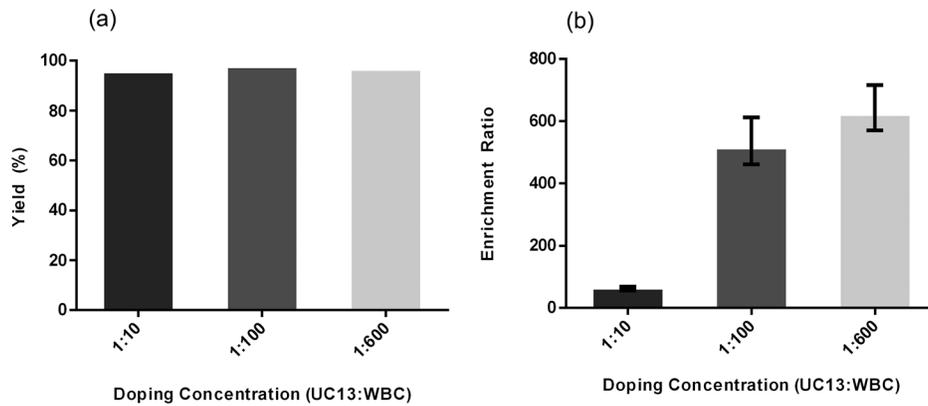


FIG. 6. Capture yields and enrichment ratio of the ratchet cell sorter at various UC13 doping concentrations.

consistent at doping ratios of 1:100 and 1:600 averaging around  $560\times$ . At the doping ratio of 1:10, however, the enrichment was found to be only  $59\times$ . This degradation in performance likely results from leukocytes occasionally becoming trapped behind UC13 cells at the entrance to a pore, preventing the leukocytes from moving up the funnel rows when high concentrations of UC13 cells are present. Therefore, at doping ratios of 1:100 or smaller, device performance is highly selective and independent of the UC13 cell concentration, which shows promise in the detection and enrichment of rare cells at diminishing concentrations.

### C. Throughput

The throughput of our cell separation device is limited by the concentration of the input sample and the size of the sorting area in the ratchet cell sorter. When cells are infused into the sorting area, the maximum amount of fluid processed in that batch corresponds to the channel volume at the bottom of the sorting area. This volume in our prototype corresponds to an hourly processing capacity of approximately 10 000 cells per hour at an input concentration of  $20 \times 10^6$  cells per ml. The throughput can be enhanced in the future through parallelization of the ratchet sorter. Specifically, if the master microstructure is fabricated on a standard 100 mm silicon wafer, we estimate this device could be parallelized to handle  $>1 \times 10^6$  cells per hour.

### D. System characterization

Finally, we evaluated the performance of the complete system that combines the hydrodynamic concentrator and the ratchet cell sorter. The sample was prepared at a concentration of  $2.5 \times 10^6$  leukocytes per ml, which is concentrated to  $10 \times 10^6$  leukocytes per ml using the concentrator. The sorting device then processed the remaining sample using operational parameters optimized previously. The average capture yield for the combined system is 97% and is independent of the UC13 doping ratio (Fig. 7(a)). The complete system shows an enrichment ratio of 822 (+198, -141) and 3000 (+643, -278) for doping ratios of 1:100 and 1:1000 UC13 cells to leukocytes, respectively. This yield and enrichment performance results in a final cancer cell purity of 89% and 75% for the two doping concentrations.

### E. Discussion

The primary advantage of this device over other filtration-based separation systems is its high selectivity, which can be evaluated using the parameters of capture yield and enrichment ratio. The capture yield of this device is 97% on average. In comparison, other filtration technologies typically report yields of 80–90%.<sup>22,25–27,30</sup> Furthermore, this yield can be obtained while simultaneously achieving high purity of the isolated sample. In our experiments where UC13 cells were spiked into leukocytes at a concentration of 1000:1, 99.7% of the contaminant leukocytes were removed, leading to a 3000 fold enrichment of the UC13 population. This

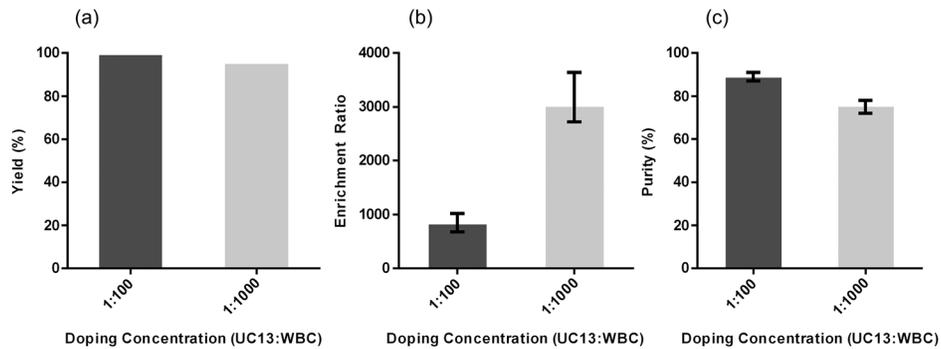


FIG. 7. Capture yield, enrichment, and purity for samples with UC13 cells doped at 1:100 and 1:1000 concentrations.

enrichment is approximately 30 times greater than that achieved in our previous work.<sup>35</sup> The capability to simultaneously achieve both extremely high yield and enrichment makes this approach promising for rare cell separation applications such as the separation of circulating tumor cells.

Another important advantage of our system is the ability to extract the separated cells and refresh the filter microstructure after each batch of cells is processed. This capability enables filtration conditions to remain constant over time. In other words, the *N*th batch of cells to enter the device is processed in the same manner as the first batch, therefore preserving high filtration selectivity. Experiments spanning several hours have shown no progressive clogging or degradation in the performance of the device over time. Furthermore, the oscillating flow reduces the time duration where each target cell is pushed against the filter microstructure to less than 30 s. In other filtration techniques, the cells remain in the pore for the entire duration of the filtration process. Reducing cell exposure to persistent forces ensures gentle handling of rare and potentially fragile cells while preserving their inherent physiological properties. Our previous work showed that there was no loss in cell viability within the ratchet device<sup>35</sup> and previous work using pinched channels similar to our concentrator demonstrate no loss in viability of the cells flowing through the pinch regions even at much higher flow rates than are used in our device.<sup>5</sup> Moreover, the ability to extract cells off the filter element makes this system cascade-able for both on-chip and off-chip downstream processing.

Finally, because our device separates cells using a combination of size and deformability, we are able to achieve better yield and purity than purely size-based separation for these two cell types. Using a cutoff of  $6\ \mu\text{m}$  ensures that even very small cancer cells are not able to pass through, while large leukocytes easily traverse through this pore into higher rows of the device. In one potential application, namely, that of separating CTCs from leukocytes, differences in deformability are likely to be a more important factor than size because of the variation in the size of CTCs<sup>48</sup> that may overlap with the size of leukocytes.

This device is easily adaptable to various cell types and applications. The concentrator will remove the same amount of fluid regardless of cell size, however, the pinch size may need to be adjusted to maintain the yield of smaller target cells. The concentrator allows samples to be prepared in larger volumes at more dilute concentrations which aids in the preparation and handling of small or delicate samples such as the sample resulting from a CD45 depletion of leukocytes. The oscillation pressure in the ratchet sorter can be adjusted lower or higher to give deformability a lesser or greater contribution in the separation process. The grouping of the funnel rows can be changed to allow more or less funnel rows to flow into the target outlet to optimize for the separation of different cell types with high selectivity.

## V. CONCLUSIONS

We used a microfluidic ratchet mechanism coupled with a hydrodynamic concentrator to separate human UC13 bladder cancer cells from leukocytes based on differences in cell size

and cell deformability. Our combined device demonstrated high selectivity with an average yield of 97% and enrichment of the cancer cell concentration by 3000 fold. The ability to selectively separate two cell phenotypes with overlapping size ranges based on size and deformability shows promise for applications where extremely sensitive separations are required for the detection of rare cells. Cells captured using our platform can be extracted easily off-chip or undergo further processing using a microfluidic platform, making this device versatile for stand-alone use or coupled with other separation technologies.

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