Streamline based design guideline for deterministic microfluidic hydrodynamic single cell traps

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A prerequisite for single cell study is the capture and isolation of individual cells. In microfluidic devices, cell capture is often achieved by means of trapping. While many microfluidic trapping techniques exist, hydrodynamic methods are particularly attractive due to their simplicity and scalability. However, current design guidelines for single cell hydrodynamic traps predominantly rely on flow resistance manipulation or qualitative streamline analysis without considering the target particle size. This lack of quantitative design criteria from first principles often leads to non-optimal probabilistic trapping. In this work, we describe an analytical design guideline for deterministic single cell hydrodynamic trapping through the optimization of streamline distributions under laminar flow with cell size as a key parameter. Using this guideline, we demonstrate an example design which can achieve 100% capture efficiency for a given particle size. Finite element modelling was used to determine the design parameters necessary for optimal trapping. The simulation results were subsequently confirmed with on-chip microbead and white blood cell trapping experiments. © 2015 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4914469>]

I. INTRODUCTION

Cells are often called the “building blocks of life” but even these basic units are complex systems in themselves. Traditionally, cells are studied exclusively *en masse* through culturing methods. However, it has become apparent over the past decade that there is actually great diversity and heterogeneity of behavior and gene expression among cells within single-type populations.¹ Population studies are useful for obtaining average cellular responses but they mask the presence of discrete sub-populations; neither are they capable of differentiating between all-or-none vs. graded responses. For instance, single cell imaging has revealed that CD4⁺ T cells exhibit threshold-triggered cytokine secretion patterns.² Higher agonist ligand concentrations increased the number of responding cells but did not increase the cytokine output of already activated cells. Single cell analysis also enables the study of many important biological processes, such as cancer initiation³ and cellular differentiation,⁴ which are inherently single cell in origin. These stem cells are typically rare and difficult to culture or isolate.

Capturing and isolating single cells for study are challenging and labor intensive with traditional laboratory equipment. Microfluidic platforms have proven to be valuable tools in this endeavor. Due to their compatible length and volume scale,⁵ they are highly amenable to single cell manipulation. Many methods of trapping single cells in microfluidic devices have been explored including hydrodynamic, optical, electric, magnetic, and acoustic.⁶ For example, Enger *et al.* employed optical tweezers to capture and move bacteria between different chambers in a microfluidic chip.⁷ Voldman *et al.* employed dielectrophoresis to trap single cells within a quadrupole electric field cage.⁸ Lee *et al.* tagged yeast cells with magnetic beads and captured them over a microfabricated electromagnet.⁹ Evander *et al.* generated standing waves with a microfabricated ultrasound transducer and trapped cells within the regions of local pressure minima.¹⁰

However, whereas the aforementioned optical, electric, magnetic, and acoustic trapping methods require additional manipulation with equipment such as lasers, electrodes, magnets, and ultrasound transducers or pre-treatment of the cells such as tagging and labelling, hydrodynamic trapping requires simply a pressure source. It is the simplest and, arguably, the most scalable cell capture technique. Consequently, many microfluidic traps are designed based on this principle. For example, Wheeler *et al.* exploited flow stagnation at T-junctions in laminar flow regimes to trap single cells for subsequent biochemical assaying but throughput was limited to a single cell per chip.¹¹ Di Carlo *et al.* demonstrated high-throughput single cell trapping and analysis by designing a sieve-like trapping array but how to determine the best trap dimensions was not discussed.¹²

In order to obtain high efficiency cell capture, the flow dynamics must be optimized. Capture efficiency is defined here as the percentage of incoming target cells captured provided that the total number of traps exceeds the number of target cells. Xu *et al.* developed a set of design criteria for a groove-shaped trap geometry which maximized trap density and single microsphere occupancy while minimizing clogging in microfluidic channels but sample use efficiency was not addressed.¹³ The most widely used design guideline for hydrodynamic trapping is that of Tan and Takeuchi.¹⁴ By modelling a trap design in terms of the flow resistances of the capturing and bypassing pathways, they were able to formulate an expression to describe the trapping efficiency. As long as the capturing flow rate was sufficiently greater than the bypassing flow rate (i.e., $Q_c/Q_b > 1$), the design was considered good. This technique has been used by many other researchers and has been shown to be reliable.^{15,16} However, while higher capturing flow rates do improve trapping efficiency, capture still remains a probabilistic phenomenon.

Here, in this paper, we present a quantitative streamline-based design method for deterministic single cell capture, which also takes into account the effect of cell size. This work differentiates itself from previous considerations of streamlines, which have only looked at them from a high-level perspective, either qualitatively¹² or semi-quantitatively,¹⁷ by defining an analytical condition for deterministic capture derived from first principles. In laminar flows, without inertial, gravitational, or collisional effects, particles will follow streamlines. This fact offers a great opportunity to deterministically direct, position, and trap particles in small volume liquids, a prerequisite for single cell analysis. The theory behind this proposal is discussed and an analytical expression is given for the minimal flow rate ratio required for 100% capture efficiency. Finite element method (FEM) and fluid-structure interaction (FSI) simulations were performed in COMSOL Multiphysics to determine the design parameters necessary to obtain the desired streamline distributions. After simulation, the trap patterns were transferred to a master mold and fabricated into polydimethylsiloxane (PDMS) chips via standard soft lithography. To validate the simulation results, solutions of polystyrene beads and white blood cells were flowed through the traps to evaluate the trapping efficiency. Finally, improvements to and further applications of these devices are discussed.

II. THEORY

A. Streamline based design guideline

A contact-based hydrodynamic trap is a structure which immobilizes a particle in a flow based on geometric confinement in a narrow region as a result of passive hydrodynamic forces. As shown in Figure 1, the whole trap consists of an inlet channel, a trap opening narrower than the size of the target particle, and one or two bypass channels. In hydrodynamic trapping, the bypass route has a higher flow resistance than the trapping route so that the moving liquid preferentially directs a target particle into the trap region if it is unoccupied. Due to the small length scales of most microfluidic systems, fluid flow is often laminar,¹⁸ wherein all fluid elements travel parallel to each other along streamlines. In general, regions of lower resistance will have higher streamline densities than regions with higher resistance. If we consider an electrical circuit analogy, lowering the resistance of one resistor in a parallel circuit increases the current going through it but does not eliminate the current going through the larger one.

FIG. 1. Schematic diagram of the streamline capturing guideline. The red line delineates the boundary between capturing (green lines) and bypassing (blue lines) regions of flow. S is the streamline cutoff, T is the threshold distance, r is the radius of a particle, Δx is a design margin indicating the minimum distance of the particle from the channel wall, w is the channel width, b is the bypass length, m is the width of the upper trap opening, n is the width of the lower trap opening, and d is the depth of the trap. Trap features are colored yellow and fluid is colored teal.

Likewise, while the majority of the flow in a channel may be directed into a trap, there still remains flow that is directed away from it as long as an alternate route exists. In order to ensure capture, the intended target particles must be prevented from entering the flow streamlines that are directed away from a trap. Therefore, the streamlines within a channel must be distributed such that the center of mass of a target particle in the flow cannot fall upon any of the bypassing streamlines. As long as the streamline cutoff, S (the thickness of bypassing streamlines measured from the channel wall), is less than the target particle radius, r , this condition is fulfilled (Figure 1). In our design, we use a slightly more relaxed threshold $T = r + \Delta x$ (Δx is a small design margin), as shown in Figure 1. This can be achieved by careful streamline distribution engineering because there is no migration of particles across streamline layers in laminar flow.

The flow velocity profile within a rectangular cross-section channel with height (h) may be expressed as a Fourier series¹⁹

$$u(x, y) = \frac{\Delta p}{2\mu L} \left\{ \left[\left(\frac{h}{2} \right)^2 - y^2 \right] - \sum_{n=0}^{\infty} a_n \cos\left(\frac{2\lambda_n y}{h}\right) \cosh\left(\frac{2\lambda_n x}{h}\right) \right\}, \quad (1)$$

where

$$\lambda_n = \frac{(2n+1)\pi}{2},$$

$$a_n = \frac{h^2(-1)^n}{\lambda_n^3 \cosh\left(\frac{\lambda_n w}{h}\right)},$$

where $u(x, y)$ is the flow velocity, Δp is the pressure drop along the channel, μ is the liquid viscosity, L is the channel length, h is the channel height, and w is the channel width. The origin of the coordinate system is the center of the channel's cross sectional rectangular shape. Integration with respect to x , along the width of the channel, and with respect to y , along the height of the channel, gives the volumetric flow rate. The capturing (Q_c^*) and bypassing (Q_b^*) flow rates for $S = T$ can therefore be expressed as follows:

$$Q_c^* = 4 \int_0^{\frac{w}{2}-T} dx \int_0^{\frac{h}{2}} dy u(x, y) = \frac{(w-2T)h^3 \Delta p}{12\mu L} \left[1 - 6 \left(\frac{h}{w-2T} \right) \sum_{n=0}^{\infty} \lambda_n^{-5} \frac{\sinh\left(\frac{\lambda_n(w-2T)}{h}\right)}{\cosh\left(\frac{\lambda_n w}{h}\right)} \right], \quad (2)$$

$$Q_b^* = 4 \int_{\frac{w}{2}-T}^{\frac{w}{2}} dx \int_0^{\frac{h}{2}} dy u(x, y) = \frac{Th^3 \Delta p}{6\mu L} \left[1 - 3 \left(\frac{h}{T} \right) \sum_{n=0}^{\infty} \lambda_n^{-5} \frac{\sinh\left(\frac{\lambda_n w}{h}\right) - \sinh\left(\frac{\lambda_n(w-2T)}{h}\right)}{\cosh\left(\frac{\lambda_n w}{h}\right)} \right]. \quad (3)$$

The same equations can be used to calculate Q_c and Q_b for any S by replacing T with S in the limits of integration. To achieve deterministic trapping, K , the ratio Q_c/Q_b for any S , must be greater than or equal to K^* , the ratio Q_c^*/Q_b^* for $S=T$

$$K = \frac{Q_c}{Q_b} \geq K^* = \frac{Q_c^*}{Q_b^*}. \quad (4)$$

This relationship can then be used to determine the necessary bypass length (b in Figure 1, which corresponds to the variable L in Eqs. (2) and (3)) to achieve $S=T$. A major assumption in calculating the bypass length is that the pressure drop across the narrowest region of the trap¹⁴ is a reasonably good approximation of the total pressure drop across the entire depth of the trap but this is not always applicable. An example showing the breakdown of this assumption is given in the supplementary material²⁰ (Example S1 and Figure S1). If a reasonably accurate estimate of the pressure drop cannot be obtained analytically, then finite element modelling can be used to determine the necessary bypass length.

B. FEM fluidic dynamics and FSI simulations

Two traps were modelled in COMSOL to illustrate unoptimized and optimized designs. The dimensions of the traps, besides the bypass length, were chosen based on the size of a nominal 10 μm diameter microsphere (Table I). The upper opening of the trap, m , was set to the diameter of the microsphere and the lower opening, n , was set to the radius. The depth of the trap feature, d , was set between one-half to two-thirds of the radius, 6 μm in this case. The channel width, w , was set to 2.5 times the diameter, 25 μm . Larger channel widths relative to the capture target diameter make it more difficult to achieve $S=T$ without an extremely long bypass length, b , while smaller channel widths increase the chances of clogging.

Laminar flow simulations were performed in 2-D with a shallow channel approximation of 15 μm in height. The inlet pressure was set to 6.89 Pa (0.001 Psi) and the outlet pressure to 0 Pa. The unoptimized design (Figures 2(a) and 2(b)) has a streamline cutoff greater than the

TABLE I. Parameters in Figure 1 for the unoptimized and optimized trap designs. r , w , m , n , d , and T are conditions imposed by the size of the particle to be trapped, while b is to be optimized.

Parameters (μm)	r	w	m	n	d	T	Δx	b	S
Unoptimized	5	25	10	5	6	6	1	113	11
Optimized	5	25	10	5	6	6	1	900	6

FIG. 2. Finite element simulations of steady state flow velocity fields for the (a) unoptimized and (c) optimized trap designs. The corresponding streamline distribution profiles are shown in (b) and (d). The red line delineates the streamline boundary. Particles traveling in the region between the two red lines will be captured and those traveling outside will be missed by the trap.

threshold distance, whereas the optimized design (Figures 2(c) and 2(d)) has a streamline cutoff equal to the threshold distance.

After simulating the fluid velocity field under laminar flow conditions, a $5 \mu\text{m}$ radius circle was added to the geometry, next to the channel wall, to model the worst-case position of a moving particle in the flow. This particle was placed $1 \mu\text{m}$ (Δx) from the channel wall to maintain sufficient mesh quality in the region between the circle and the channel wall. This gap is reasonable considering a particle is less likely to be in direct contact with the wall while in motion. Transient FSI simulations were run using the previously determined stationary laminar flow velocity field as the initial conditions (see source code and Figures S2 and S3 in the supplementary material).²⁰ However, because the streamline distribution changes slightly when a particle is added to the flow, it was placed $0.5 \mu\text{m}$ pass the streamline cutoff in the simulation of the optimized design.

III. EXPERIMENTAL

A. Materials

UltraPure distilled water was purchased from Invitrogen (Grand Island, NY). Phosphate buffered saline (PBS) with EDTA was purchased from Celprogen (San Pedro, CA). Cross-linked poly(styrene/divinylbenzene) non-functionalized microspheres ($9.65 \mu\text{m}$) were purchased from Bangs Laboratories (Fishers, IN). Tween-20 and Fluorinert FC-40 were purchased from Sigma-Aldrich (St. Louis, MO). SU-8 3010 was purchased from MicroChem (Westborough, MA), and AZ 9260 was purchased from AZ Electronic Materials (Branchburg, NJ). Cal-Lyse lysing solution was purchased from Life Technologies (Carlsbad, CA). Tygon tubing (0.020" ID) was purchased from Cole-Parmer (Vernon Hills, IL). Syringe needles (23 G) were purchased from BD (Franklin Lakes, NJ).

B. PDMS chip design and fabrication

PDMS microfluidic chips were fabricated using conventional multi-layer soft lithography.²¹ Design layouts were drawn in AutoCAD and printed onto either chrome (Photo Sciences, Inc., Torrance, CA) or transparency (CAD/Art Services, Bandon, OR) masks.

To fabricate the master molds, photolithography was performed on silicon wafers. For the trap layer, SU-8 3010 was spun onto a 3" silicon wafer at 1100 rpm for 30 s ($H = 18\ \mu\text{m}$), soft baked at 95°C for 30 min, exposed with an *i-line* mask aligner at $756\ \text{mJ}/\text{cm}^2$, post exposure baked (PEB) at 95°C for 5 min, developed for 6 min in SU-8 developer, and hard baked at 155°C for 5 min. For the flow layer, AZ 9260 was spun on the same 3" wafer at 1300 rpm for 30 s ($H = 16\ \mu\text{m}$), soft baked at 110°C for 5 min, rehydrated for 30 min, *i-line* exposed at $1617\ \text{mJ}/\text{cm}^2$, developed for 4 min in AZ 400 K 1:3 developer, and reflowed at 130°C for 1 min. For the control layer, the steps were the same as for the AZ flow layer.

To fabricate the chips, PDMS was cast into the molds. First, RTV615 was mixed at 5:1 (A:B) ratio for 5 min, degassed, poured onto the control mold, and placed into a 75°C oven. Meanwhile, RTV615 was mixed at 20:1 ratio for 5 min, poured onto the trap/flow mold, spun at 2500 rpm for 60 s, and placed into a 75°C oven. The control layer was par-baked for 1 h, while the trap/flow layer was par-baked for 45 min. Next, the control PDMS was peeled off the mold, cut into individual chips, aligned onto the trap/flow layer, and returned into the oven for 1 h and 30 min. Then, the 2-layer device was peeled off the mold and baked overnight. Finally, inlet/outlets were punched with a 0.75 mm biopsy punch and bonded to a glass slide via air plasma treatment (Electro-Technic Products, Model BD-20 V).

Before use, the control lines were filled with FC-40 to prevent air from being forced into the flow channels during actuation. The flow/trap layer was then filled with buffer (1% Tween-20 in water) to degas the channels and render them hydrophilic. The overall chip is shown in Figure 3(a) with color-coded flow and control channels. The top two inlets were used to flow in buffer and micro-beads. Based on the relative pressure between the inlets, the beads could be positioned along the channel width. The direction of flow was controlled by actuating the control valves as required. The lower left hand side of the chip was for adjusting the particle position and the lower right hand side for testing the traps. Each chip design consisted of five consecutive traps (Figures 3(b) and 3(c)).

C. Bead preparation and trapping

Microspheres were diluted in 1% Tween-20 water in a microcentrifuge tube to approximately $10^5/\text{ml}$ and vortexed before use to ensure dispersity. The tube caps were punctured with 23 G needles to form a pressure inlet and a sample outlet. Pressure was applied to the inlet to push the bead solution towards the tip of a needle-fitted Tygon tube forming a droplet. This

FIG. 3. Light microscopy images of (a) the PDMS chip with control channels in red and flow channels in blue, (b) close-up of the unoptimized trap, and (c) close-up of the optimized trap. The left side of the chip in (a) is used to control the position of the beads/cells in the flow. White arrows indicate direction of flow.

droplet was then merged with a droplet of buffer on the surface of the chip from the pre-filling of the channels. The merging of the droplets prevented the introduction of air bubbles into the system when switching tubing. Beads were then loaded into the chip via gravity driven flow (~ 700 Pa or 0.1 Psi), and bead trapping was recorded by a video microscope (Figures S4 and S5 in the supplementary material).²⁰

Solutions with known bead counts ranging from 1 to 5 were prepared by trapping a certain number of beads in the optimized traps and then recovering them into a $50\ \mu\text{l}$ volume of buffer. Due to the elastomeric properties of PDMS, the trapped beads were able to be forced through the traps at high pressure into an outlet tube for collection. This tube was then unplugged from the outlet port and inserted into the bead inlet port. The collected sample was then flowed into the chip to determine the capture efficiency by comparing the known number of beads in the solution to the number of beads actually captured.

D. White blood cell preparation and trapping

Whole blood ($2\ \mu\text{l}$) was collected from a healthy subject into a microcentrifuge tube and immediately mixed with $20\ \mu\text{l}$ of $1\times$ PBS with EDTA to prevent clotting. To remove the red blood cells, Cal-Lyse lysing solution ($20\ \mu\text{l}$) was added to the blood sample, mixed, and incubated at room temperature for 10 min in darkness. Next, deionized water ($100\ \mu\text{l}$) was added to the sample, mixed, and re-incubated for another 10 min. Then, $350\ \mu\text{l}$ of distilled water was added to the sample, mixed, and re-incubated for another 10 min. Afterwards, the tube was centrifuged for 5 min at 300 g. The supernatant was removed and the sample was re-suspended in $100\ \mu\text{l}$ of PBS with EDTA and mixed. Centrifugation and re-suspension were repeated until the supernatant was clear. Assuming a typical white blood cell count of $5\times 10^6/\text{ml}$, the final white blood cell density is on the order of $10^5/\text{ml}$. We have observed empirically that this density is sufficiently low enough to minimize the chances of multiple cells from simultaneously entering the trap region while maintaining a reasonable trapping time. The white blood cell suspension was loaded into the chip as described for the microspheres and trapping was recorded by a video microscope (Figure S6 in the supplementary material).²⁰ All mixing was performed by manual pipetting.

IV. RESULTS AND DISCUSSION

A. Bead trapping

Under very low Reynolds number laminar flow, we expect moving particles to remain in the streamlines that they are traveling on. The simulation results were verified in the observed trajectories of the beads both in the unoptimized and optimized designs. For the unoptimized design, the bead ($v\approx 480\ \mu\text{m/s}$) diverged away from the first trap as predicted by simulation (Figures 4(a) and 4(b)). Interestingly, the bead also bypassed all subsequent traps. Because of the laminar flow regime, poorly designed traps placed directly in series with each other such as in this design do not increase the chances of capture. Once a bead misses the first trap in a series, it will miss all subsequent traps because the streamline distribution remains the same. The only way that a bead could be captured in this design was for it to ride along a streamline no farther than $1.5\ \mu\text{m}$ from the exact center of the flow. The effective range of the streamlines upon which a $10\ \mu\text{m}$ bead can travel is from its radius to half the channel width (i.e., $5\ \mu\text{m}$ to $12.5\ \mu\text{m}$ as measured from the channel wall). Assuming that the probability of a bead to be found within this range is uniformly distributed, this corresponds to a 20% chance of capture.

For the optimized design, the bead ($v\approx 270\ \mu\text{m/s}$) diverged initially at the mouth of the trap but then converged back into the trap as predicted by the streamline pattern in the simulation (Figures 4(c) and 4(d)). This suggests larger viscous than inertial forces (i.e., low Reynold's number) as expected under laminar flow. In order to be lost in such a design, a bead must be pushed into an adjacent bypassing streamline at the mouth of the trap but due to the physical barrier of the channel wall, the bead will be forced into the capturing streamlines at the next trap. The fact that even beads traveling along the edge of the channel, the most

FIG. 4. COMSOL FSI simulation of a $10\ \mu\text{m}$ bead in (a) unoptimized and (c) optimized designs. Superimposed time-lapse images of the corresponding experimental results are shown in (b) and (d). White arrows indicate direction of flow.

unlikely candidates for capture, suggests that this trap design can capture all incoming beads regardless of their position in the flow. Further experiments using solutions with exact bead counts demonstrated a 100% capture efficiency in the optimized design (Figure 5). The trapping process also proved to be very robust since alternating the flow direction over multiple cycles did not cause the bead to be lost into the bypass channel once it was captured. The total time to completely fill all the traps was less than 5 min. Faster loading may be easily achieved by using higher pressures. Slower flow rates were used in this work for the purpose of capturing videos with a conventional, low frame rate camera.

B. White blood cell trapping

To assess the suitability of this design for biological cells, which are deformable and not perfectly spherical, we tested the traps with human white blood cells. Like the beads, the white

FIG. 5. Capture efficiency of the optimized trap design using buffer solutions with an exact bead count from one to five ($n = 3$). Sample volume was $50\ \mu\text{l}$. All beads are captured individually at different trap locations (i.e., no multiple trapping at one location).

blood cells ($v \approx 220 \mu\text{m/s}$) also followed the streamline trajectories and were focused into the trap (Figure 6(a)). The cells appeared intact and undamaged by the trapping process (Figure 6(b)). Retention of cell integrity is critical to cell viability and reliable single cell experiments. Unlike beads which are characterized by a rigid shell, cells have flexible membranes which can deform, especially at high flow velocities. One way to control the flow rate in a pressure driven system without changing the pressure gradient is to partially actuate one or multiple control valves that are across the channel to increase the overall flow resistance, thereby reducing the velocity. Tuning of the resistance can be achieved by varying the percentage closure of each valve.

Another difference between beads and cells is in their size distributions. Microspheres are generally manufactured to have high uniformity in size but white blood cells cover a much wider breadth, from approximately $6\text{--}20 \mu\text{m}$ in diameter. In order to capture a range of particle sizes, the traps must be designed according to the radius of the smallest expected target. If a trap can capture particles with radius r_0 , then it will also capture particles with radius greater than r_0 , provided that the channel can accommodate their size without clogging. However, there is a limit to how wide a range can be reasonably accommodated without extremely long bypass channels.

In practice, some important experimental parameters such as cell density, cell size distribution, the number of trap sites, and flow velocity may affect the capture efficiency. High cell density may lead to channel clogging thus reducing the capture efficiency. Non-uniform cell size distribution can lead to loss of smaller cells if the traps are not designed to trap them. An insufficient number of trap sites can lead to overflow. Flow velocity should not affect the capture efficiency as long as the flow remains in the laminar regime but high pressure across the trap can lead to deformation of cells which may cause them to squeeze through. However, all these effects can be remedied by either design change or careful control of experimental conditions such as reducing the cell concentration, optimizing the trap design for the smallest target cells, increasing the number of traps, and limiting the driving pressure, respectively.

C. Improvements and future work

A common problem encountered in both the bead and cell trapping experiments was the presence of large dust particles and/or cellular debris clogging the traps. This can be remedied by integrating an on-chip filter composed of PDMS posts spaced at predetermined intervals upstream of the trap region; preferably near the inlet.²² These can be patterned onto the master flow mold during fabrication and casted simultaneously with the flow layer.

Due to the small volume of the bypass channels (less than 0.5 nL in the optimized design), these traps show great promise for conducting highly sensitive cellular assays with low dilution

FIG. 6. (a) Superimposed time-lapse image of white blood cell trapping and (b) close-up of captured white blood cell. The white arrow indicates the direction of flow.

factors. Combined with elastomeric on-chip valves,²¹ arbitrary manipulation of captured single cells may be performed such as redirection of a selected cell to a reaction chamber for further analysis. However, the large footprint of each individual trap due to the long bypass channels may be undesirable for high density trapping. One way to circumvent this is to design a single pair of bypass channels, which starts at the beginning of the first trap and terminates at the end of the last trap. This streamline design guideline may also be applied to trapping arrays.^{12,23,24} By optimizing the design such that all relevant streamlines are captured, multiple targets of interest may be captured in a high-throughput manner without any loss provided that the number of traps exceeds the number of expected targets.

V. CONCLUSION

In summary, we have provided a quantitative streamline-based design guideline for hydrodynamic single cell traps with theoretically 100% capture efficiency on a microfluidic platform as evidenced by bead/cell capture in an optimized design even at a worst case position (i.e., adjacent to the channel wall). In contrast to past works, this technique presents a novel approach to trap design that provides a definite, quantitative condition for deterministic capture. By taking into consideration the target particle size, higher capture efficiency may be obtained compared to optimizing flow rates alone. With the growing interest in and importance of single cell study, the design guideline outlined in this paper provides a means of maximizing the capture efficiency of scarce biological samples such as circulating tumor cells or embryonic stem cells. We believe that the application of this technique towards cellular immunoassays and trapping arrays is especially promising and will prove to be advantageous for highly sensitive detection of single cell protein levels and high efficiency rare cell capture, respectively.

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- ¹J. M. Raser and E. K. O'Shea, *Science* **309**, 2010 (2005).
- ²J. Huang, M. Brameshuber, X. Zeng, J. Xie, Q. Li, Y. Chien, S. Valitutti, and M. M. Davis, *Immunity* **39**, 846 (2013).
- ³R. Bjerkvig, B. B. Tysnes, K. S. Aboody, J. Najbauer, and A. J. A. Terzis, *Nat. Rev. Cancer* **5**, 899 (2005).
- ⁴S.-I. Nishikawa, L. M. Jakt, and T. Era, *Nat. Rev. Mol. Cell Biol.* **8**, 502 (2007).
- ⁵R. Milo, P. Jorgensen, U. Moran, G. Weber, and M. Springer, *Nucleic Acids Res.* **38**, D750 (2010).
- ⁶J. Nilsson, M. Evander, B. Hammarström, and T. Laurell, *Anal. Chim. Acta* **649**, 141 (2009).
- ⁷J. Enger, M. Goksör, K. Ramser, P. Hagberg, and D. Hanstorp, *Lab Chip* **4**, 196 (2004).
- ⁸J. Voldman, M. L. Gray, M. Toner, and M. A. Schmidt, *Anal. Chem.* **74**, 3984 (2002).
- ⁹H. Lee, A. M. Purdon, and R. M. Westervelt, *Appl. Phys. Lett.* **85**, 1063 (2004).
- ¹⁰M. Evander, L. Johansson, T. Lilliehorn, J. Piskur, M. Lindvall, S. Johansson, M. Almqvist, T. Laurell, and J. Nilsson, *Anal. Chem.* **79**, 2984 (2007).
- ¹¹A. R. Wheeler, W. R. Throdsset, R. J. Whelan, A. M. Leach, R. N. Zare, Y. H. Liao, K. Farrell, I. D. Manger, and A. Daridon, *Anal. Chem.* **75**, 3581 (2003).
- ¹²D. Di Carlo, N. Aghdam, and L. P. Lee, *Anal. Chem.* **78**, 4925 (2006).
- ¹³X. Xu, P. Sarder, Z. Li, and A. Nehorai, *Biomicrofluidics* **7**, 014112 (2013).
- ¹⁴W.-H. Tan and S. Takeuchi, *Proc. Natl. Acad. Sci.* **104**, 1146 (2007).
- ¹⁵S. Kobel, A. Valero, J. Latt, P. Renaud, and M. Lutolf, *Lab Chip* **10**, 857 (2010).
- ¹⁶J.-P. Frimat, M. Becker, Y.-Y. Chiang, U. Marggraf, D. Janasek, J. G. Hengstler, J. Franzke, and J. West, *Lab Chip* **11**, 231 (2011).
- ¹⁷L. Lin, Y.-S. Chu, J. P. Thiery, C. T. Lim, and I. Rodriguez, *Lab Chip* **13**, 714 (2013).
- ¹⁸T. M. Squires and S. R. Quake, *Rev. Mod. Phys.* **77**, 977 (2005).
- ¹⁹H. A. Stone, in *CMOS Biotechnology*, edited by H. Lee, R. M. Westervelt, and D. Ham (Springer US, 2007), pp. 5–30.
- ²⁰See supplementary material at <http://dx.doi.org/10.1063/1.4914469> for videos of the FSI simulation results and on-chip trapping experiments.
- ²¹M. A. Unger, H.-P. Chou, T. Thorsen, A. Scherer, and S. R. Quake, *Science* **288**, 113 (2000).
- ²²J. T. Nevill, R. Cooper, M. Dueck, D. N. Breslauer, and L. P. Lee, *Lab Chip* **7**, 1689 (2007).
- ²³D. Wlodkowic, S. Faley, M. Zagnoni, J. P. Wikswo, and J. M. Cooper, *Anal. Chem.* **81**, 5517 (2009).
- ²⁴A. M. Skelley, O. Kirak, H. Suh, R. Jaenisch, and J. Voldman, *Nat. Methods* **6**, 147 (2009).