HIGH-THROUGHPUT CELLULAR SAMPLE PREPARATION VIA ULTRAFAST SOLUTION EXCHANGE
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ABSTRACT
Sample preparation is a critical process in analytical methods, but it is often challenging to integrate into microfluidic systems. The primary tasks in sample preparation involve solution exchange. Here, we take advantage of inertial focusing equilibrium positions to transfer cells and particles from one solution to another in as fast as 2 ms with a throughput compatible with downstream flow cytometry. We demonstrate the method’s efficacy with a range of representative applications from a simple solution exchange about cancer cells to isolating white blood cells from hypotonically lysed red blood cells.

KEYWORDS: Inertial Microfluidics, High-Throughput, Single-Cell, Sample Preparation

INTRODUCTION
The manipulation of cells, particles, and fluid is a critical function of miniaturized systems which aim to replicate and automate the function of macroscale laboratory techniques (centrifugation, washing, labeling, etc.) or carry out previously unattainable operations on cells. For example, processes in hematologic analysis include lysing red blood cells, labeling cells with antibody probes, and washing away unbound probes[1,2]. Numerous challenges have arisen when attempting to translate these simple tasks between micro- and macroscale systems due to differences in physical principles which can be applied at these scales. To automate this process would greatly enhance the number and complexity of assays which could be performed. An ideal microsystem for automated sample preparation and single-cell analysis will operate in continuous flow, with a high-throughput, continuously removing unbound probes from the cell suspension, requiring no externally applied forces, and yielding cells precisely positioned in flow for inline interrogation. Previously, we identified the important design criteria for high-throughput inertial focusing to align particles to precise positions in a flow[3]. In this work, we apply these criteria toward the development of a miniaturized system for solution exchange about cells and evaluate it with several critical applications.

THEORY
This novel microfluidic system sequentially manipulates inertial focusing equilibrium positions to rapidly transfer particles and cells from one solution to another (Figure 1). Briefly, suspended cells are injected at a high flow rate (Reynolds Number≈100) and migrate to a geometrically determined dynamic equilibrium position. The channel converges with another channel carrying an exchange solution at a higher flow rate; a new equilibrium position is geometrically defined to lie within the exchange solution in the established coflow. Due to the high Peclet number diffusion is negligible for the length of the channel. Cells migrate laterally into the exchange solution in as fast as 2 ms and exit the channel immersed in the exchange solution, where they are repositioned for inline measurements.

Figure 1: System schematic. A. Computer-aided design drawing of the top view of the microfluidic channel (channel height = 30 µm). B. Schematic of the solution transfer mechanism. C. Numeric solution of inertial lift forces acting on a 10 µm sphere in one quadrant of a high aspect ratio microchannel. Lift forces direct cells to dynamic equilibrium positions centered at the long faces of the channel.
EXPERIMENTAL

All microchannels were fabricated by bonding molded polydimethylsiloxane (PDMS) to glass slides. Molds for PDMS were made using standard photolithographic methods. Solutions and cell and particle suspensions were injected through polyetheretherketone (PEEK) tubing into the channel by syringe pumps. A numerical simulation of cross-sectional lift forces was performed in COMSOL Multiphysics using a custom script for a Lagrangian specific flow field. All imaging was performed with a Phantom v7.3 high speed camera mounted on an inverted microscope. The concentration of the exchange solution was quantified in channel outlets by plate reader absorbance measurements. Polystyrene microspheres were used to characterize the size dependence of lateral migration velocity. Whole human blood was mixed with red blood cell lysis buffer when demonstrating the removal of red blood cell debris. MCF7 cells were harvested from culture and bound to 1 µm streptavidin coated dynabeads via biotinylated anti-EpCAM.

RESULTS AND DISCUSSION

We first evaluated the system by quantifying size-dependent lateral migration velocities of polystyrene beads as they migrated into a solution of trypan blue (employed in experiments for its contrast with buffer) and determined the solution exchange efficiency (Figure 2A-B). 97% of beads above 15 µm in diameter were transferred while the transfer solution remained 100% pure (ratio of absorbance in collection outlet to inlet). With these empirical guidelines we selected common laboratory sample preparation tasks to demonstrate the utility of the technology. First, we demonstrated the transfer of MCF7 cells (breast cancer epithelial cell line) from buffer to trypan blue—similar in outcome to a fixed-volume centrifugation and resuspension (Figure 2C).

Next, we showed that leukocytes from whole blood mixed with red blood cell lysis buffer can be rapidly transferred to a new solution (Figure 3A). The contents of red blood cells and their debris can confound analytical measurements; this method will be useful as an automated wash step upstream of analyzers like flow and impedance cytometry. We also demonstrated the separation of unbound microbeads from cell-bound beads as part of a cell isolation procedure (Figure 3B). This will enhance microfluidic immunomagnetic devices whose efficiency is limited by adverse behavior of unbound beads.

Figure 2: Device characterization. A. Overlayed high-speed images of particles of different sizes migrating laterally. B. Dependence of lateral migration speed on particle size. C. Overlayed high-speed images of an MCF7 cell migrating laterally.

Figure 3: Applications of high-throughput solution exchange. A. Illustration and high-speed images of solution exchanged about leukocytes in lysed blood. B. Illustration and high-speed images of solution exchanged about 1-µm bead coated MCF7 cells in a suspension of coated MCF7 and free beads.
Lastly, while flow cytometry can usually discriminate between free and bound fluorescent probes, the study of low affinity interactions requires higher concentrations of free probes which hinder the accuracy of the flow cytometer in this task[4]. With this ultrafast solution exchange system we will transfer probe-bound objects to a new solution with low background and immediately record fluorescence measurements. Such an integrated system will extend the utility of flow cytometry to the study of previously inaccessible low affinity binding interactions.

CONCLUSION
We have introduced a platform technology capable of high-throughput cellular sample preparation which manipulates inertial lift forces to transfer cells from one solution to another. We have demonstrated its utility in several application-oriented experiments and envision its adoption as a modular expansion to a flow cytometer or integrated into a specialized analysis system.

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REFERENCES

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