INERTIAL MICROFLUIDIC BAND-PASS SEPARATIONS
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ABSTRACT
This work presents an inertial microfluidic band-pass filter, enabling continuous separation of three sizes of particles or cells with <3 μm difference in diameter. The design uses inertial forces to focus particles into two bands along side walls. Two chambers with side outlets are cascaded in series to generate microvortices for continuous band-pass separations. Particles with diameters 23 μm, 18.5 μm and 15 μm were used as cell surrogates to demonstrate separation. Sorting of particle aggregates, doublets, and single particles was also demonstrated. Our results suggest that this simple planar device is applicable for continuous separation of cell aggregates, doublet and single cells.

KEYWORDS: Inertial microfluidics, cell separations, band-pass separations

INTRODUCTION
Inertial microfluidics uses hydrodynamic forces acting on cells to position them in the flow [1,2]. These forces drive cells to migrate across streamlines and order in equilibrium positions based on their size, leading to passive, label-free cell separation, purification and enrichment. Applications in cell separation [1], flow cytometry [3], and rare cell enrichment [4] have been demonstrated, achieving passive cell manipulation with high throughput.

While inertial microfluidics has achieved size-based separation of particles and cells with high throughput [1,2], most designs only have a single cut-off size for bipolar selection. Our group has reported spiral devices for separation of three different-sized particles [1], but challenges with outlet design, device integration and need for >5 μm size-difference limit their applications. Herein, we present an inertial microfluidic system based on vortex-aided separation. Using particles as cell surrogates, we demonstrated band-pass separations of three different-sized particles or aggregates with precise size control. This is extremely advantageous for separating cells of interest from smaller non-target cells and larger cell aggregates.

DESIGN PRINCIPLE
Using the two-stage focusing model [5], we designed a high aspect-ratio microchannel (50 μm x 100 μm, w x h) to order particles into two bands along channel side walls. Two microchambers were located in series for continuous separation of particles (Fig. 1a). In the high aspect-ratio channel, particles experience shear lift force F_s and wall lift force F_w that balance each other leading to inertial focusing. By designing the microchannel length into 10 mm, 20 μm diameter particles can fulfill first stage focusing into two bands along the sidewalls which is the optimal condition for efficient extraction by laminar vortices [4].

In the chamber, the flow is separated into main flow, sheath flow and vortex. As particles enter the chamber region, the absence of the channel wall disrupts the force balance. Thus the lateral migration of particles is dominated by the inertial shear lift force across the streamlines toward the vortices. Larger particles experience larger inertial force and migrate further into sheath flow, while smaller particles remain in main flow fulfilling separation. By cascading two microchambers and designing proper chamber dimension and channel flow resistance, the inertial microfluidic system can serve as a band-pass filter separating large, medium, and small particles from different outlets (Fig. 1b).

EXPERIMENTAL
We used standard soft-lithography methods to fabricate the polydimethylsiloxane (PDMS) - based inertial microfluidic devices. In all the experiments, we injected fluorescent particles (Polyscience inc.) into the devices using a syringe pump (NE-1000, New Era Pump Systems, Inc.). To take fluorescent vortex images or particle stream images, we used an inverted epi-fluorescence microscope (IX71, Olympus Inc.) equipped with a 12-bit high-speed.
CCD camera (Retiga EXi, QImaging). We overlay 20 images and added pseudo-colors to form fluorescent particle-stream pictures in ImageJ. To exhibit trajectory of particle in bright-field, we used the same equipment while setting the exposure time to minimum value (10 µs) and sequentially took 300 images with minimum time interval. We stacked 300 images in ImageJ to establish a complete view of particle motion. We used a hemocytometer (Hausser Scientific) to measure the concentration of inlet and outlets and calculate the separation efficiency.

RESULTS AND DISCUSSION

To enable successful two-stage separations in this cascaded system, flow resistances and chamber geometries have to be matched to provide proper flow condition for each chamber and tuned to the cut-off sizes for separations (Fig.2a). Flow resistance ratio of side and main channels influences flow separation and the boundary streamline (Fig. 1b, red dashed line). The position of the boundary determines the lateral migration distance for particles to enter the sheath flow, thus leads to different cut-off size for separation. We designed a 500×500µm² chamber with flow resistance ratio $R_1/R_c=8$ as the upstream chamber. Using flow rate $Q_{in}=500µL/min$, this chamber has 21µm upper cut-off size. Based on analogous electric circuit model, the presented microfluidic system can be simulated as paralleling of resistors (Fig.2b). The flow (current) is separated into different outlets (branch circuit) depending on the resistance ratio. Thus $Q_{in}=500µL/min$ with $R_1/R_c=8$ leads to $Q_c=400µL/min$. For such flow rate, we designed a 500×1000µm² chamber with $R_2/R_c=10$ as the downstream chamber with 17µm lower cut-off size. Thus, the inertial microfluidic band-pass filter has 4µm pass-band from 17µm to 21µm.

As a proof-of-concept, we used microparticles as cell surrogates and separated a mixture of 23µm, 18.5µm and 15µm diameter particles. The stacked bright-field images ($N=300$) illustrate >23µm diameter particles were extracted from O1 following the sheath flow, while 18.5µm and 15µm diameter particles were separated downstream into O2 and O3 (Fig.3a). We should note that some particles were observed circulating in the vortex areas in both chambers. However the motions of particles were distinct. In the upstream chamber, particles recirculated in the vortices for a short period of time and exit through the side outlets. While in the downstream chamber, a few particles were trapped

![Figure 2](image1.png)

**Figure 2:** The flow resistance network and chamber geometry are modified to tune the cut-off size in each chamber. (a) The flow resistance network. (b) Analogous electric circuit model.

![Figure 3](image2.png)

**Figure 3:** (a) Separation of >23 m, 18.5 m and 15 m diameter particles from different outlets. (b) Samples from inlet and outlets indicate successful separation. The white arrows indicate >23 m diameter particles. (c) The histograms illustrate the size distribution of particles in inlet and outlets, indicating successfully filtration of 18.5 m diameter particles from O2 with 90% purity. The purities of 23 m and 15 m diameter particles are also enhanced clearly after separation. (d) The concentration 18.5 m diameter particles are enriched 2.5×. (e) Normalized count illustrates that the separation efficiencies of 23 m, 18.5 m and 15 m diameter particles are 67%, 63% and 93% correspondingly.
inside the vortices due to the large vortex area. The fluorescent images of particles in inlet and outlets illustrate successful separation of these particles based on size (Fig.3b).

More quantitatively, the histograms exhibit particle size distribution in inlet and outlets indicating successful separation and purification (Fig. 3c). As particles travel downstream the inertial microfluidic band-pass filter, the purity of 23µm diameter particles extracted from O1 increases from 8% to 59% compared to inlet. Similarly, the purity of 18.5µm diameter particles increases from 69% to 90% after band-pass filtration from O2. The 15µm diameter particles elute from O3 with purity increasing to 62%. The concentration plot also indicates a 2.5× enrichment in concentration of 18.5µm diameter particles after band-pass filtration (Fig.3d). Moreover, the normalized count shows that the separation efficiencies for 23µm, 18.5µm and 15µm diameter particles are 67%, 63% and 93% respectively (Fig.3e). The efficiency could be further improved by optimizing flow conditions and channel resistances.

In addition to the band-pass separation of spherical particles, the system can also sort particle aggregates based on aggregate size. Though particle aggregates are non-spherical, different type of aggregates have distinct equivalent sizes. As shown in Fig. 4, aggregates of five have largest equivalent size and are extracted by high-pass filter, while doublets and single cells are sorted by the band-pass and the low-pass filters correspondingly. These results suggest that continuous separation of cell aggregates, doublet cells and single cells is possible with small differences in size. This ability may be further applied in sorting specific subpopulations of cells from heterogeneous biological samples in cell biology research.

CONCLUSIONS

In conclusion, we have successfully demonstrated an inertial microfluidic band-pass filter that enabled continuous separation of three different-sized particles or aggregates with ~3µm size-difference. Unlike bipolar selections in previous inertial microfluidic designs, the presented device provides new band-pass separation functionality thus can be used for more complicated separation tasks. We believe this platform will open new opportunities in the microfluidic field for selective separation of particles or cells within the designed pass-band in a wide range of applications in particle synthesis and purification, as well as cell biology research.

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