Electronic Supplementary Material (ESI) for Lab on a Chip

Automation of Cellular Sample Preparation Using a Centrifuge-on-a-Chip

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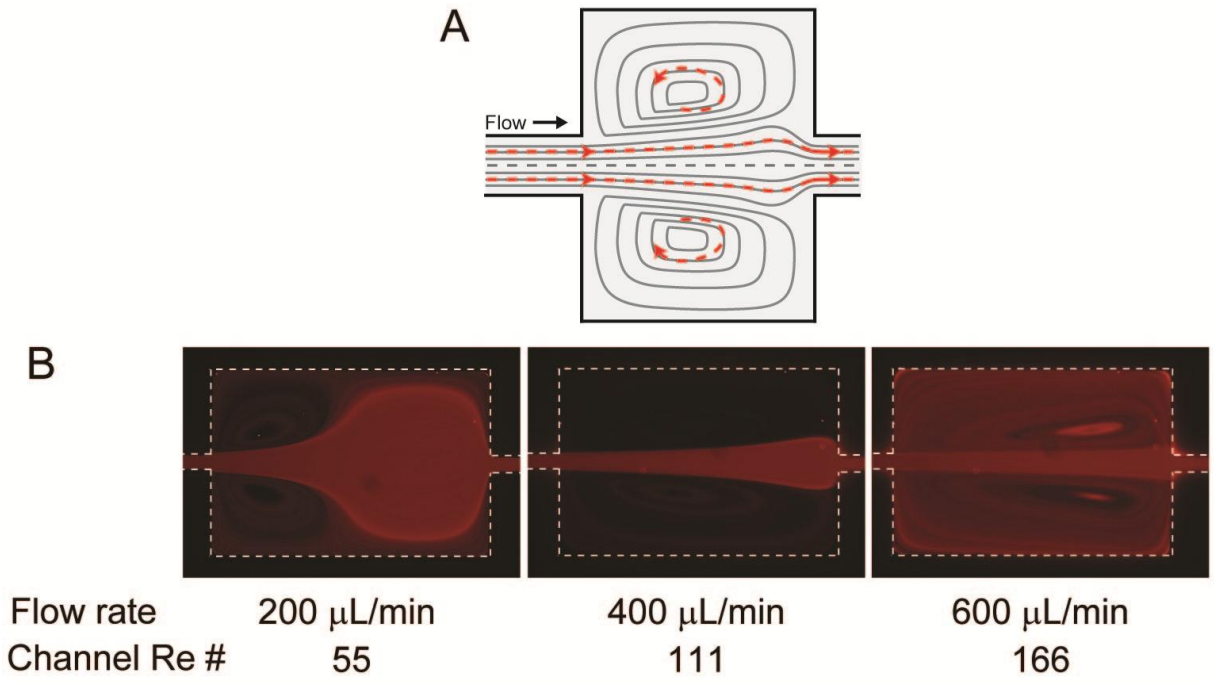
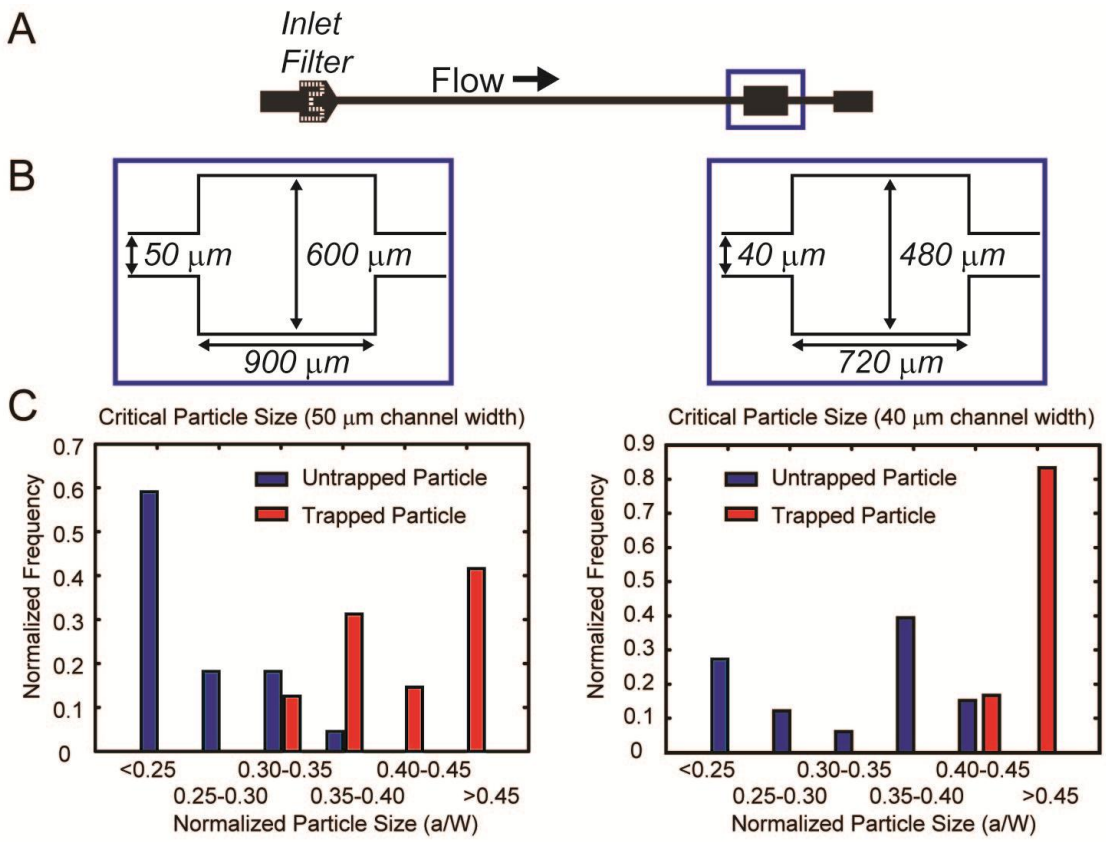


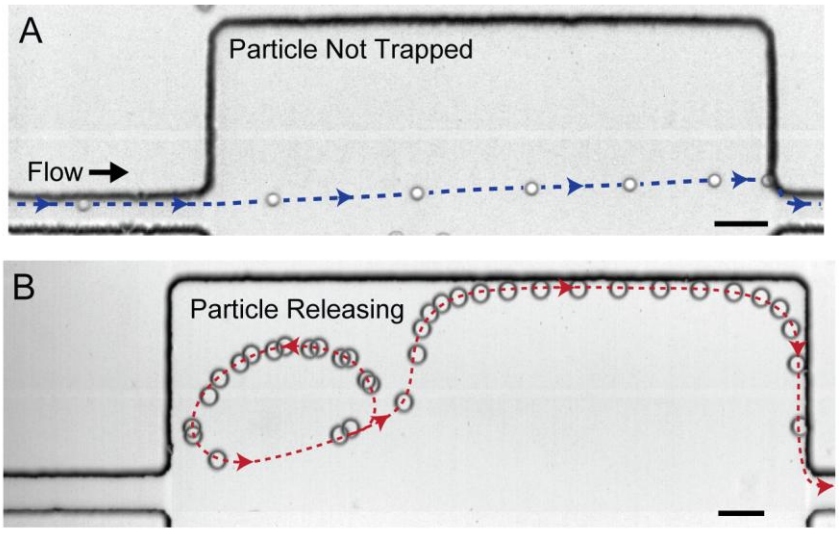
Supplementary Figure 1

Microvortex formation in expansion chambers. (A) Schematic of fluid streamlines (gray) with direction of flow (red-dotted line) in expansion chambers. (B) Fluorescent 1 μm particles trace the fluid streamlines at various channel Reynolds number, R_c. The vortex size increases to occupy the entire chamber with increasing R_c.



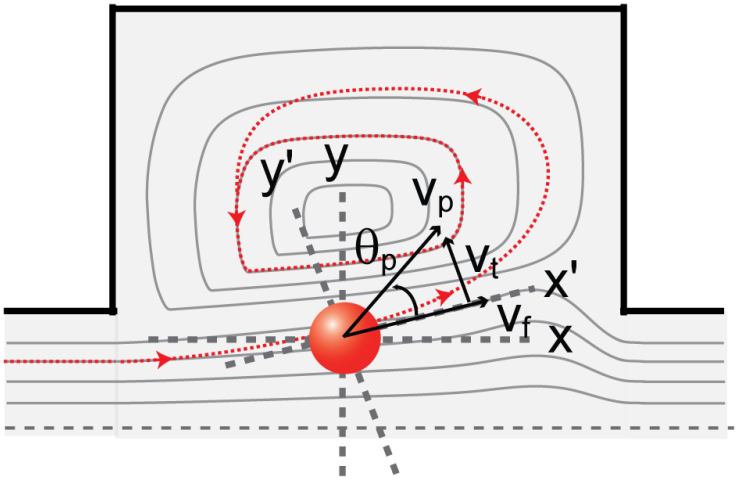
Supplementary Figure 2

Critical particle size for trapping. (A) Schematic of single channel device comprised of inlet filter region, straight channel region and an expansion chamber (boxed in blue). (B) Two device designs for the expansion chamber were used (C) Particle trapping was observed for a range of particle diameters between a/W=0.3-0.4 for a 50 μm channel width and 0.4-0.45 for a 40 μm channel width. Particles smaller than the critical size were incapable of being trapped and flowed past the trapping chamber and out of the system. Interestingly, these results suggest that the critical size leading to entry into the vortex does not depend strongly on the inlet channel width.



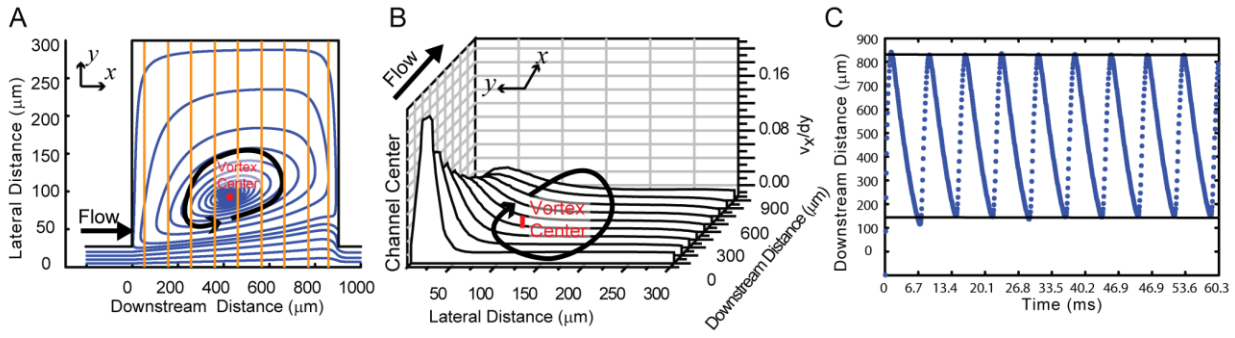
Supplementary Figure 3

Particle entry and release from vortices. (A) A trajectory for a smaller particle that is not trapped in the microvortex is shown. The particle flows out of the system because it experiences a smaller lift force and follows fluid streamlines that pass the trapping chamber (B) A trajectory for a particle being released from a vortex trap. The particle is released from the vortex trap by decreasing the flow rate. The vortex becomes smaller in size resulting in the particle being released to the main flow.



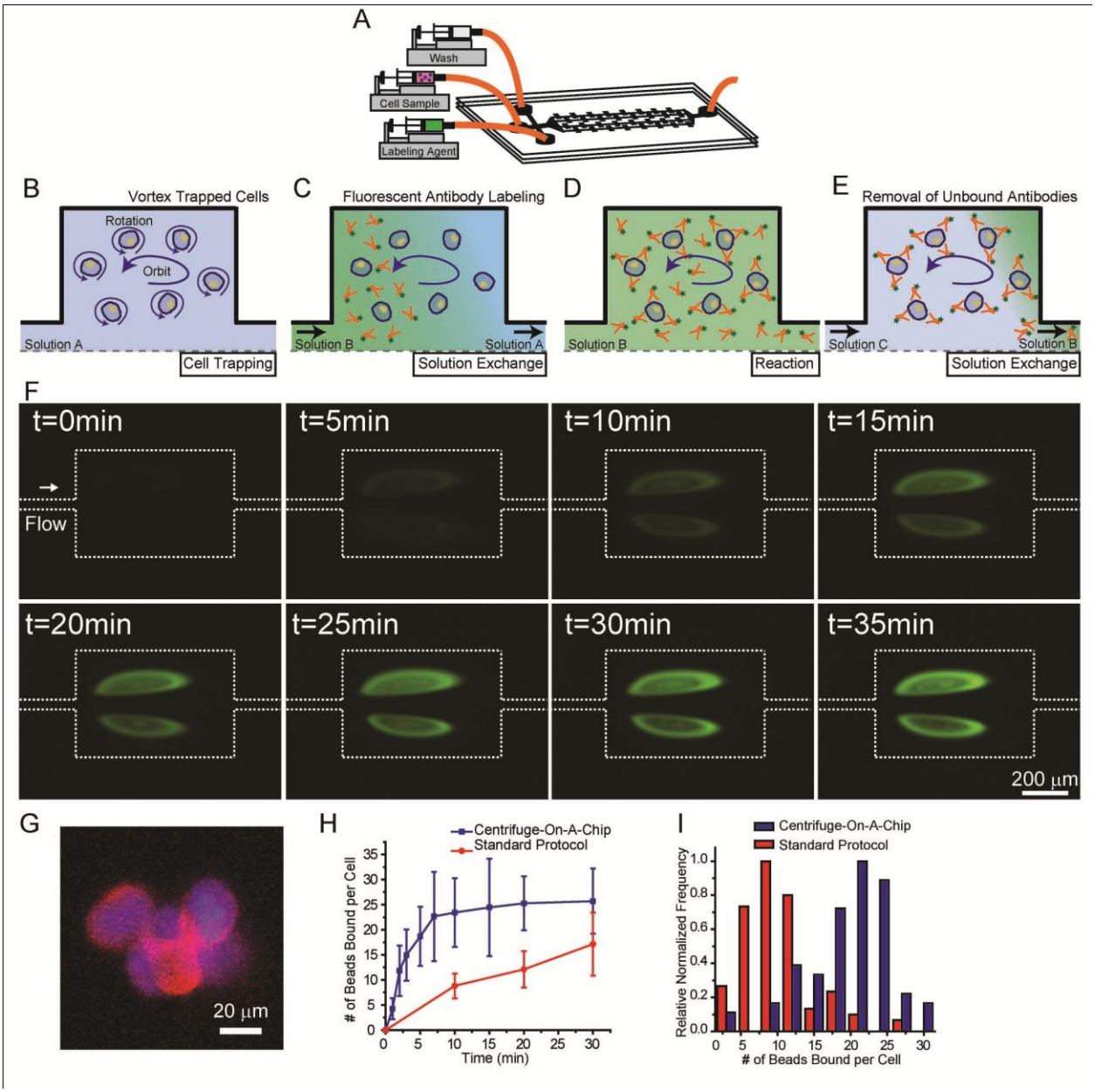
Supplementary Figure 4

Calculation of the shear-gradient lift force. With the absence of the wall, the particle experiences a dominant shear gradient lift force that drives the particle through fluid streamlines into the vortex. This lift force can be measured by calculating the cross-streamline velocity of the particle, v_t. The instantaneous cross-stream velocity is accompanied by a Stokes drag that is equal in magnitude but opposite in direction of the lift force at steady state: $F_L = 3\pi\mu a v_t$, where μ is the fluid viscosity, a is the particle diameter, and v_t is the transverse velocity which is calculated based on the mismatch in particle velocity and fluid velocity at each time point.



Supplementary Figure 5

Shear rate and flow in microvortices. (A) Using COMSOL finite element simulations, slices of velocity in the downstream direction (orange) were analyzed to determine shear rate. Shear rate is important to determine the strength of shear gradient lift. (B) The shear rate was mapped over the expansion-contraction chamber. The maximum shear rate decreases as the fluid flows downstream and is significantly lower inside the vortex than in the channel center. The gradient in shear rate also decreases further downstream along the vortex, suggesting that lateral migration would be strongest near the upstream portion of the vortex. (C) The unique velocity field also affects the dynamics of particle orbits: the particle accelerates when it is closer to the channel center and slows down near the outer edge of the vortex.



Supplementary Figure 6

On-chip cell labeling. (A) Schematic of the experimental setup with three syringe pumps containing cell sample, labeling reagent, and a PBS wash solution. (B) Unlabeled MCF7 cells are injected into the device and 'trapped' while continuously rotating and orbiting inside the microvortex. (C) Solution is exchanged to flow fluorescent antibodies and allow (D) continuous 3D reaction. (E) Once cells are labeled, PBS solution is introduced to wash the cells and remove unlabeled antibodies. Cells can then be released from the vortex traps into a collection vial. (F)

Fluorescent streak images of MCF7 cells incubated with Calcein AM orbiting inside the fluid vortex for > 30 min. Fluorescence intensity increases over time as more Calcein AM is intracellularly converted to fluorescent Calcein. This experiment also demonstrates the ability for long term maintenance of trapped cells necessary for long incubations with reagents without cell loss. (G) A fluorescent image of a cluster of cells that was sequentially trapped inside the fluid vortex, fixed with paraformaldehyde, permeabilized, labeled with anti-Cytokeratin-PE & DAPI, and released into a 96-well-plate for observation. (H) MCF7 cells covered with biotinylated anti-EpCAM are coated with streptavidin conjugated microbeads in < 5 min at the same level as a standard off-chip protocol after 30 min. (I) After 30 min, both methods demonstrate uniform labeling with microbeads over the cell population.

Spiked (Input)		Recovered (Output)			Metrics		
Cancer cell	RBC & CD45+	Cancer cell	RBC	CD45+	Efficiency (%)	Enrichment	Purity (%)
146	~2,500,000,000	31	-	-	21.2	-	-
146	~2,500,000,000	25	-	-	17.1	-	-
146	~2,500,000,000	27	-	-	18.5	-	-
423	~2,500,000,000	93	-	-	22.0	-	-
423	~2,500,000,000	88	-	-	20.8	-	-
423	~2,500,000,000	93	-	-	22.0	-	-
972	~2,500,000,000	119	39	-	12.2	7851178	75.3
972	~2,500,000,000	189	197	-	19.5	2468594	48.9
972	~2,500,000,000	157	231	-	16.2	1748800	40.4
342	~2,500,000,000	89	498	3	28.7	1431563	16.4
342	~2,500,000,000	72	203	5	21.0	2533327	25.7
342	~2,500,000,000	84	137	4	24.6	4359958	37.3
				AVG	***20.3***	***3398902***	***40.7***
				STDEV	***4.17***	***2406423***	***20.5***

Supplementary Table 1

Counts for spiked and recovered cancer and blood cells. Calculated metrics include efficiency, enrichment, and purity.

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Supplementary Movies

Supplementary Movie 1

Particle trapping in a microvortex. This movie shows a single PDMS particle entering and remaining trapped in a vortex observed with high speed video microscopy. Once trapped, the particle continuously orbits in an elliptical path within the vortex reaching a stabilized position. Channel dimensions: 70 μm height, 50 μm channel width, 600 μm and 900 μm chamber size. Total real time of movie ~8 ms.

Supplementary Movie 2

Separate orbits for a pair of different sized particles trapped in a vortex. Larger particles orbit closer to the vortex center. Channel dimensions: 70 μm height, 40 μm channel width, 480 μm and 720 μm chamber size. Total real time of movie ~30 ms.

Supplementary Movie 3

Particle release from a microvortex. This movie shows a single PDMS particle released from the vortex trap observed with high speed video microscopy. This process was accomplished by reducing the input flow rate which decreased the fluid vortex size. Channel dimensions: 70 μm height, 50 μm channel width, 600 μm and 900 μm chamber size. Total real time of movie ~2-3 ms.

Supplementary Movie 4

Enrichment of cancer cells from human blood. This movie demonstrates the sequential steps of loading a spiked dilute blood sample (20% blood) through the device, flushing of the blood cells

out of the device, and releasing the cancer cells from the vortex trap for downstream collection. Total real time of movie ~40 s. Typical experiments take < 3 min to process a 10 mL blood sample.

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Supplementary Methods

1. Microfluidic device fabrication and setup. Devices were fabricated using standard soft lithography techniques. Briefly, KMPR 1050 (Microchem) was spun at 1800 rpm for 30 s to create a 70 μm-thick layer on a 10-cm silicon wafer. The pattern was photolithographically defined in this layer by using a Mylar mask printed at 40,000 dpi. After development, polydimethylsiloxane (PDMS) (Sylgard 184 Dow Corning Corp.) was poured onto the photoresist master at a 10:1 ratio of base to crosslinker, degassed in a vacuum chamber, and cured at 65^{o}C overnight. The devices were then cut from the mold, ports were punched with a punch kit (Technical Innovations), and the devices were bonded to glass slides using oxygen plasma for 30 s (Harrick Plasma). After plasma treatment and placement onto the glass substrate, the devices were maintained at 65^{o}C in an oven for 15 min to increase bonding.

PDMS microchip devices were mounted onto the stage of an inverted fluorescent microscope (Nikon TE2000-U). Two inlet ports of the device were connected to two syringe pumps (Harvard Apparatus PHD 2000) by PEEK tubing (Upchurch Scientific) while an outlet port was routed into a waste container or collection tube. One syringe pump contained saline solution for washes, while another syringe pump contained the bead or cell solution.

2. Experiments with single PDMS particles in fluid vortices. Polydisperse PDMS (Polydimethylsiloxane) beads were prepared using an emulsion polymerization process. PDMS was mixed at the standard 10:1 ratio of resin to crosslinker (Dow Corning; Sylgard 184), degassed, and added to deionized water containing 0.1% Tween 20 at 10% w/v PDMS. The uncured solution was mixed vigorously with a vortexer and placed in an oven overnight at 65^{o}C to allow hardening into solid PDMS beads. After curing, PDMS beads smaller than 50 μm were extracted from the bead solution via centrifugation. Any remaining beads larger than 50 μm were removed via filtration through a filter upstream of the trapping arrays in the device.

Polydisperse PDMS particles with sizes (a/W=0.1-0.7 and particle diameter ranging from ~1 to ~35-50 μm) were injected (average velocity = 2.8 m/s) into two different vortex trapping chips with 70 μm channel height. One device had a 50 μm channel width and a vortex chamber of 600 μm width by 720 μm length, while the other device had a 40 μm channel width and a chamber of 480 μm width by 560 μm length. Using high-speed video microscopy, we tracked

the trajectories of single PDMS particles as they migrated and stabilized in the fluid vortices. Individual particle trajectories were visualized and recorded with Phantom v7.3 high-speed camera (Vision Research, Inc.) conducted with 60-100 μs times between frames.

The particle trajectories were mapped using a custom MATLAB program that determined the PDMS particle center automatically. The automated program converted each raw image to a binary image where channel walls and PDMS particles form defined intensity peaks. Each image frame was subtracted from a background image, which contains only the channel walls, leaving a defined intensity peak representative of the particle. The program measured the particle diameter and located the particle center position, which was then normalized to the channel center and orifice of the expansion-contraction chamber. This process was repeated for each frame until all images were analyzed. Each video consisted of ~1000 frames, which was long enough to observe the particles migrating into and become stably trapped within a fluid vortex. Particles were recognized to be stably trapped when the orbiting trajectory within the vortex remained constant. Particles that were not trapped in fluid vortices contained < 10 frames for image analysis. Figures demonstrating particle migration trajectories and particle orbits within the vortex included data binned according to particle size. Each particle range (n = 3 or more samples) contained particle positions that were averaged, mapped, and overlaid onto fluid streamlines computed using finite element modeling. To determine the magnitude of shear gradient lift force, F_L, that each particle experiences, the inertial lift was assumed to balance Stokes drag on a sphere $F_L = 3\pi\mu a v_t$ where the force is dependent on the particle diameter a and transverse velocity v_t. This transverse velocity is derived from the mismatch in particle and fluid element trajectories as the particle migrates across streamlines.

3. Cell line preparation. MCF7 breast cancer cells (ATCC) were cultured in media containing DMEM supplemented with 10% FBS, 1% bovine insulin, and 1% penicillin/streptomycin (Invitrogen). Cells were typically passaged every 4 days. Single and clustered cells were made by incubation for less time with trypsin, 1-2 minutes, in room temperature before neutralizing trypsin with media and resuspending cells in PBS. Blood samples were obtained by venous puncture of healthy human volunteers by a trained physician. Blood was collected in EDTA tubes and used within 48 h.

4. Cell spiking and staining. Experiments involving rare cell isolation required diluting blood with PBS to 5% to 20% v/v followed by spiking of known amounts of cancer cells. Cancer cell number and concentration was determined by transferring a 100 μL volume of cell solution using a micropipette into a 96-well microtiter plate. After waiting for10 min for the cells to sediment, three wells were imaged using fluorescence microscopy to count the number of cells. The average value was taken as the cell spiking concentration. 100 μL of cell solution was spiked into each blood sample for cell capture efficiency, enrichment and purity experiments described below. Typically, there were about ~500 cells spiked in each 0.5 mL of whole human blood diluted with 9.5 mL PBS. Capture efficiency, enrichment and purity experiments required pre-labeling cancer cells with fluorescent dye (CellTracker Blue, Invitrogen). Blood samples were incubated with CD45-FITC (Invitrogen).

5. Experiments with isolating cancer cells from blood. The PDMS device contained 8 parallel channels with two inlets and one outlet. One inlet was connected to a syringe containing diluted blood spiked with cancer cells while the second inlet was connected to a syringe containing PBS as a 'wash'. The spiked dilute blood sample with a total volume of 10 mL was injected through the device at a flow rate of 4.4 mL/min. Once the blood sample was processed, PBS was introduced into the device by switching syringe pumps from the blood sample solution to the PBS solution, maintaining a flow rate of 4.4 mL/min. This flush step was necessary to remove untrapped blood cells in the main flow and in the vortex traps. Cells trapped in the vortex were subsequently released by stopping the syringe pump carrying PBS, and collected in a 96-well-plate. This collection step also required lowering the pressure in the blood inlet to prevent additional forward flow by disengaging the syringe from the pump.

Cells were imaged after collection into the smaller volume on the well plate to collect information about capture efficiency, enrichment, and purity. Each well of a 96-well plate was used to collect captured cells from one blood sample and contained ~200 μL liquid volume. After allowing ~10 min for the cells to sediment in the well, each well was imaged using a Photometrics CoolSNAP HQ2 CCD camera mounted on a Nikon Eclipse Ti microscope. The whole well was automatically imaged in a few minutes (4X and 10X objective) using an ASI motorized stage operated with Nikon NIS-Elements AR 3.2 software. Captured images were automatically obtained for four configurations: brightfield, FITC, TRITC and DAPI filter sets.

Collected images were automatically stitched together using the NIS-Elements Software. Images were analyzed by enumerating the number of cells present in the well. Capture efficiency is defined as the ratio of the number of target cells captured in the 96-well-plate to the number of cells spiked into dilute human blood. Enrichment is defined as the ratio of target cancer cells to contaminant cells in the output divided by the same ratio in the inlet. Purity is defined as the ratio of selected target cancer cells captured to the total number of captured cells. Data are reported as mean ± standard deviation of the mean as noted.

6. Experiments with on-chip cell labeling. Labeling of cells on chip was conducted with a PDMS device containing 4 parallel channels with multiple inlets for introducing cells, PBS wash, and labeling agent(s) and one outlet. Unlabeled MCF7 cells were first flowed into the system at 2 mL/min and trapped in vortices where they were maintained in orbits around the vortex center. Trapped cells were then introduced to labeling agents via rapid solution exchange by switching syringe pumps from the cell solution to the labeling agents and maintaining the overall flow rate. Cells were 'trapped' for extensive periods of time from 15 to 30 minutes while exposed to the labeling agents for reaction(s) to take place. Upon completion of labeling, PBS was used to provide a quick wash step to remove unbound labeling agents and for on-demand release of cells into a 96-well-plate. Each experiment contained ~1000 labeled cells.

All labeling agents were spiked in 30 mL PBS and introduced into the device at a flow rate of 2 mL/min via a 30 mL syringe connected to a syringe pump. Fluorescent agents include Calcein AM (Invitrogen, intracellular fluorogenic stain activated by esterases, 12 μL for 15 min), FITC-conjugated anti-human anti-EpCAM (CD326, Biolegend, cell surface antibody stain, 25 μL for 15 min), DAPI (Invitrogen, nucleic acid stain, 6 μL) and FITC-conjugated anti-human anti-Cytokeratin (Sigma, intracellular antibody stain that requires fixation and permeabilization agents, 6 μL), and primary mouse anti-human anti-EpCAM (CD326, eBiosciences, cell surface antibody stain, 20 μL for 15 min) and secondary of goat anti-mouse Alexa Fluor 647 (Invitrogen, cell surface antibody stain, 10 μL for 15 min). The latter two labeling approaches required four total syringe pumps for introducing two labeling agents. For cells labeled with intracellular antibody stains, cells trapped in vortex were first fixed with 1% paraformaldehyde for 10 min, and subsequently permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) and aforementioned labeled agents in PBS for 15 min. In experiments for labeling cells with beads, cells were

incubated with biotinylated anti-human anti-EpCAM (CD326, eBiosciences, 10 μL for 30 min) in PBS outside of the device, injected into the chip, and sequentially exposed to 1 μm streptavidin-conjugated Dynabead® solution (Invitrogen, 20 μL in 50 mL) for 30 min. Cells labeled with beads were analyzed via high-speed microscopy while orbiting in vortex traps.

To characterize the performance of labeling on-chip, we conducted parallel control experiments using standard protocols with a benchtop centrifuge. Control experiments were prepared with a 15 mL centrifuge tube using the same parameters used for the Centrifuge-on-a-Chip. This included labeling ~1000 cells with the same amounts of labeling reagents in 1 mL of PBS and incubation for the same amount of time. Cells prepared with the standard protocol were washed after each labeling step with a standard benchtop centrifuge. The cells were then placed in a well of a 96-well-plate and imaged using brightfield and fluorescence microscopy (Nikon NIS-Elements AR 3.2).

For on-chip fluorescent labeling of isolated cancer cells from blood, a similar experimental setup was used with four syringe pumps. Blood spiked with cancer cells was introduced into a parallel device until the sample was processed, 'washed' with PBS, fixed with 1% paraformaldehyde for 10 min, and permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) and labeled agents in PBS for 15 min. Labeling agents include DAPI (Invitrogen, nucleic acid stain, 6 μL) and PE-conjugated anti-human anti-Cytokeratin (Sigma, intracellular antibody stain that requires fixation and permeabilization agents, 6 μL). Labeled cells were then released into a 96-well-plate for observation.