Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

High-Recovery Sorting of Small Lung Cancer Cells from Whole Blood via a Periodic-Focusing Inertial Microchip

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Supporting material contents:

Supplementary Video 5. White blood cells (WBCs) and lung cancer cells (A549 cells) flowed in the inertial microfluidic channel near the outlets at 609 μL/min flow rate.

Supporting information section 1: blood lysing protocol.

Commercial whole blood was gently shaken to be homogeneous prior to use. For lysing erythrocytes, 900 μL distilled water was added to 100 μL whole blood for 30 s. Immediately afterward, 110 μL of 80 g/mL NaCl solution was added to restore osmolarity. Blood mixture was then centrifuged at 4500 rpm for 5 min, supernatant was discarded to remove lysed erythrocytes.

Supporting information section 2: flow cytometry analysis.

Flow cytometer (FACSCalibur, Becton-Dicknson) was used for particle distribution analysis after particle characterization experiments. All particles were detected directly without any further modification. Original samples and sorted samples collected from all outlets were analyzed by counting at least 10,000 cells.

Supporting information Table S1: Chip parameters.

	Parameters
Radius	500 μm
Width	240 μm
Height	50 μm
Total length	5.5 cm
contractions	110 μm

Figure S1. ANSYS fluent mesh images.

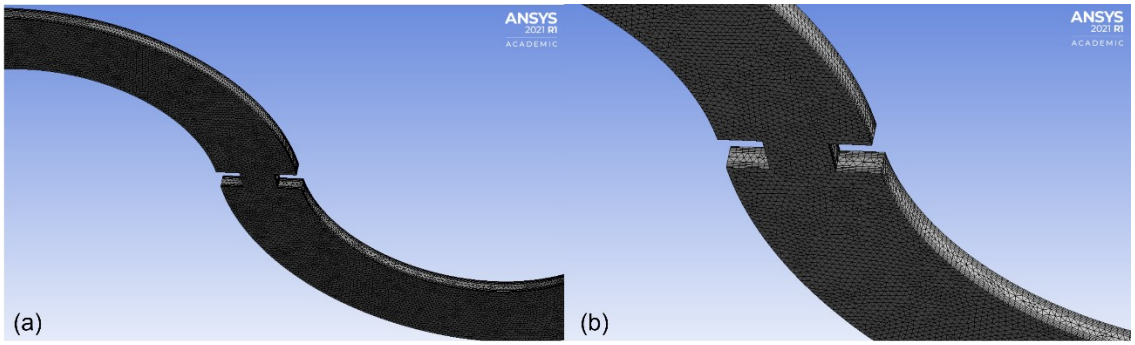

Figure S1 graph (a) illustrates the ANSYS fluent meshes near contractions and graph (b) is magnifying meshes near one contraction for visualization purpose.

Figure S2. Schematic illustration for separating rare tumor cells from whole blood.

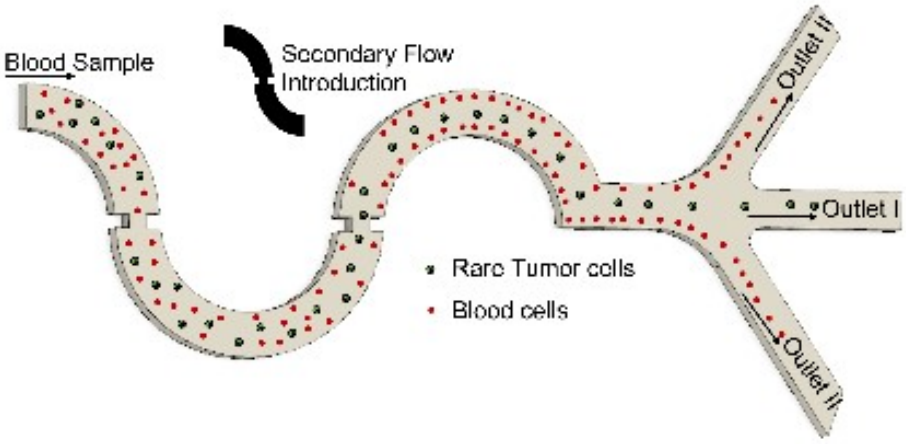

Figure S2 demonstrates the trajectories of two cells (rare tumor cells and blood cells) at Outlet I and Outlet II. Green particles which represent tumor cells will be focused in the middle of the channel

after passing through the serpentine channel and exit from Outlet I and Red particles which represent red blood cells will be focused next to the side of the channel and flow out from Outlet II.

Figure S3. Flow rate optimization.

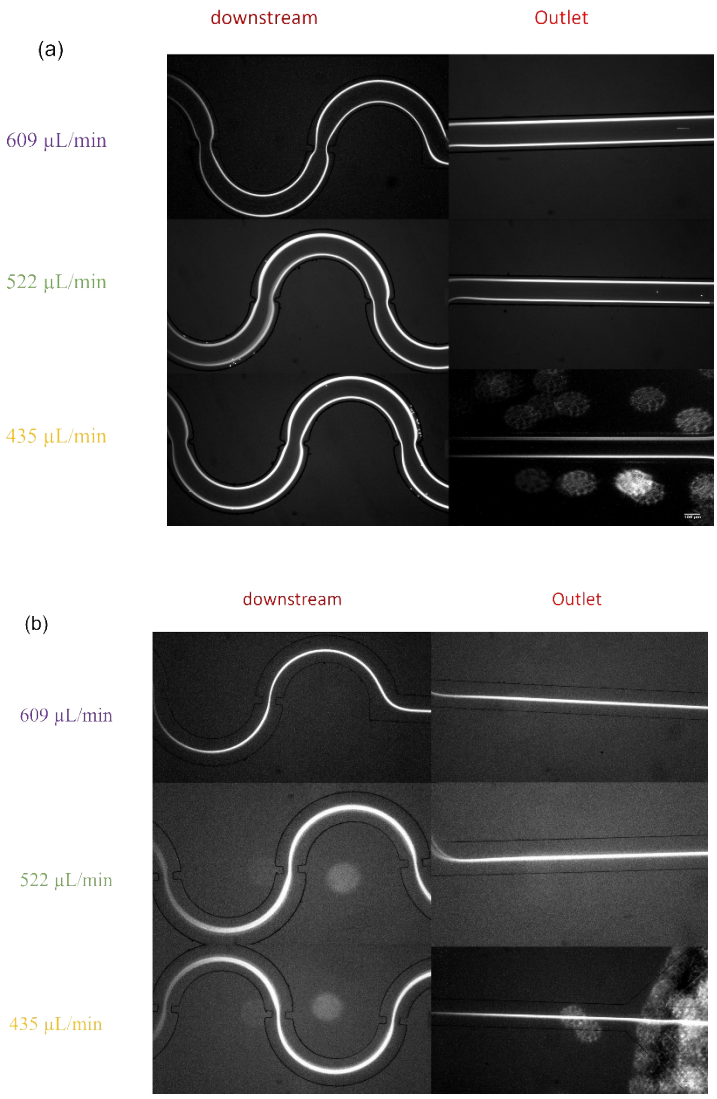

Figure S3 (a) Fluorescence images of 4 µm particles at representative positions. (b) Fluorescence images of 13 µm particles at different positions. Scale bar is 100 µm.

Flow rate optimization was studied using 4 and 13 µm polystyrene beads by using flow rate 435 µL/min, 522 µL/min , and 609 µL/min which corresponding to the Reynolds number 50, 60

and 70. No differences in trajectories could be observed under microscope for 4 or 13 µm polystyrene beads for different flow rate. The back pressure resulted in some of the chip leaking when 698 µL/min ($R_e = 80$) was applied. Therefore, considering the throughput cancer cells can stand and chip leaking, 609 µL/min was chosen for following tumor cell study.

Figure S4. Cell viability control.

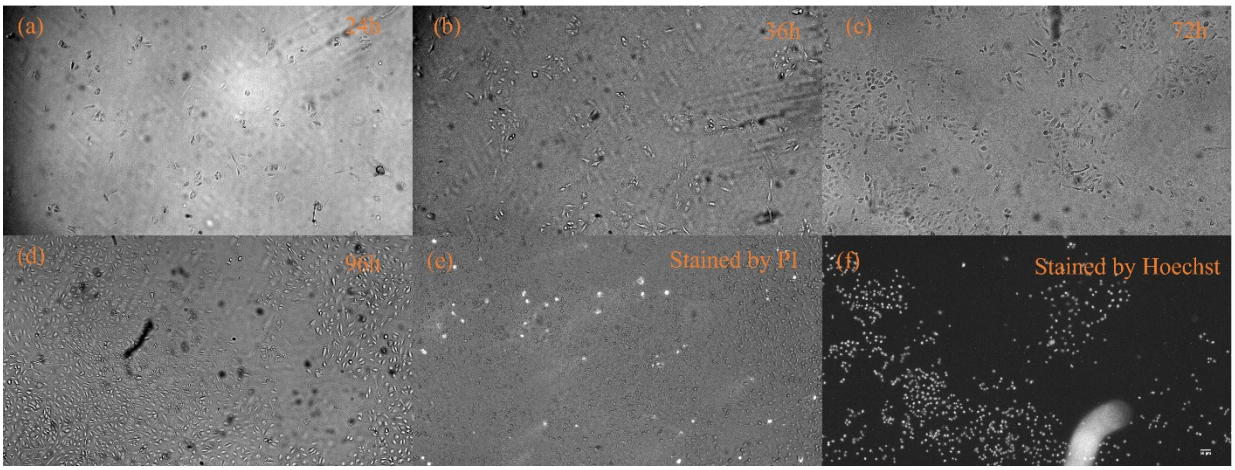

Figure S4. (a-d) Microscopic images of the re-cultured tumor cells 24, 36, 72 and 96 h. (e) 96h cultured A549 cells were stained with PI and fluorescence cells are dead cells. (f) 96 h cultured A549 cells were stained with Hoechst to calculate cell viabilities (same position with picture taken for graph (e)). As shown graph e and f, 94.2% of the cells maintained the desired proliferation capacity and viability after cultured 96 h under the same condition described in method part.

References

1. Nath, B.; Raza, A.; Sethi, V.; Dalal, A.; Ghosh, S. S.; Biswas, G., Understanding flow dynamics, viability and metastatic potency of cervical cancer (HeLa) cells through constricted microchannel. *Scientific reports* **2018,** *8* (1), 1-10.

2. Moon, H.-S.; Kwon, K.; Hyun, K.-A.; Seok Sim, T.; Chan Park, J.; Lee, J.-G.; Jung, H.-I., Continual collection and re-separation of circulating tumor cells from blood using multi-stage multi-orifice flow fractionation. *Biomicrofluidics* **2013,** *7* (1), 014105.
3. Connolly, S.; McGourty, K.; Newport, D., The in vitro inertial positions and viability of cells in suspension under different in vivo flow conditions. *Scientific reports* **2020,** *10* (1), 1-13.