Supporting Information

Isolating Rare Cells and Circulating Tumor Cells with High Purity by Sequential eDAR

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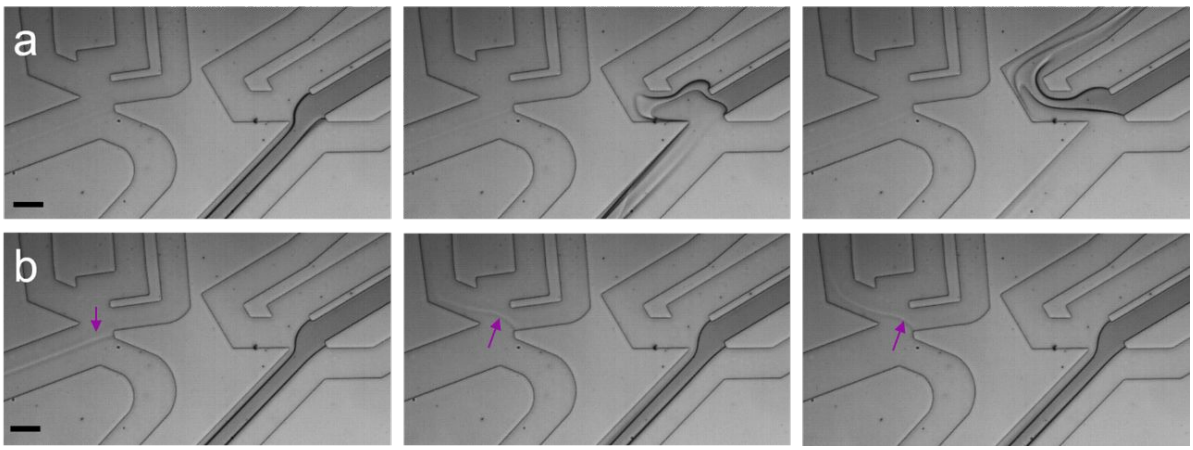

Figure S1. Sequential sorting fluidics scheme. The visualized flow was a mixture of 30% glycerol solution (25% glycerol in Isoton II buffer) and 70% green food dye. **a** One sorting event at the first junction (right side of images). From left to right: no solenoid open; first solenoid was triggered, flow shifts left; flow fully switched to the channel connecting the two sorting junctions. **b** One sorting event at the second junction (left side of images), while the first solenoid was switched off. From left to right: no solenoid open; second solenoid was triggered, flow shifts left; flow fully switched to the collection channel. The aliquot at the second sorting junction was more difficult to visualize, indicating the aliquot was diluted further after passing through the first sorting junction. The purple arrows were used to mark flow at the second sorting junction. Scale bars are 200 μm.

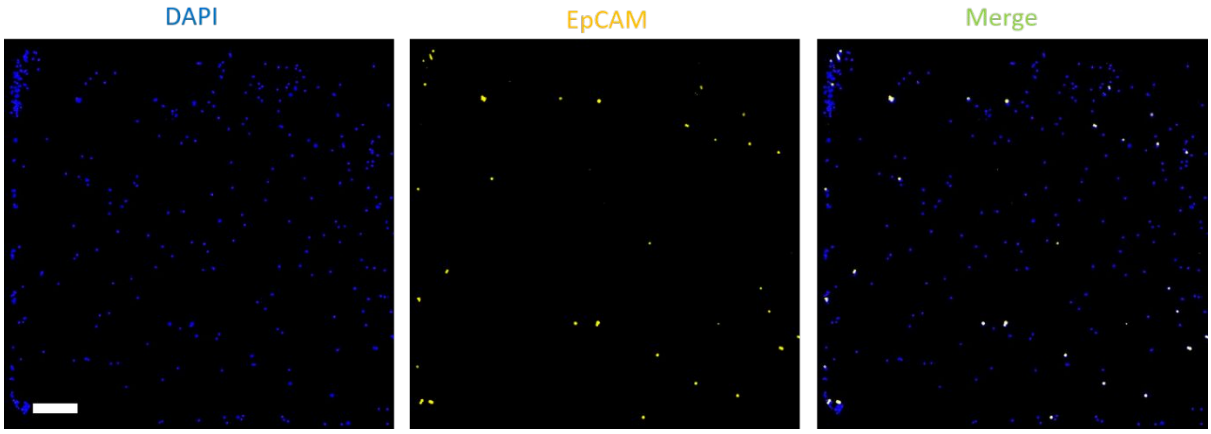

Figure S2. Fluorescent images of collected cells after sorting with a 2-stage 3 cm-channel chip. DAPI (blue) channel showed cell nuclei of collected cells and PE-EpCAM (yellow) channel showed MCF-7 cells. The scale bar is 500 μm.

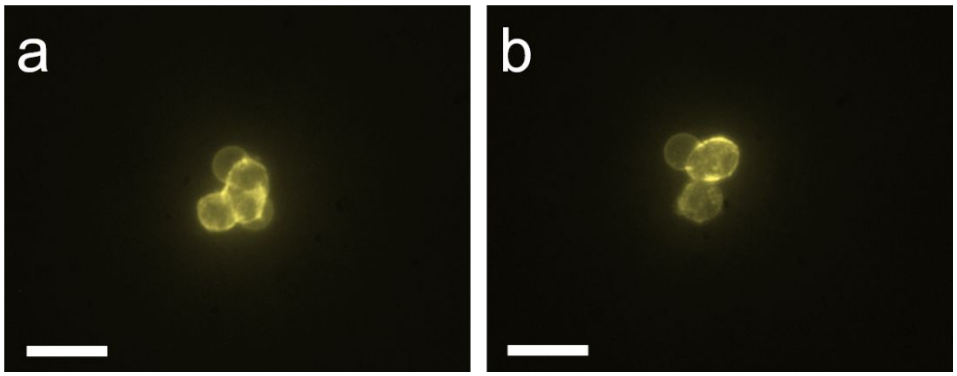

Figure S3. a,b Two clusters of MCF-7 cells. MCF-7 cells were labeled with PE-anti-EpCAM and sorted into a multi-well plate using a sequential eDAR chip. Scale bars are 40 μm.

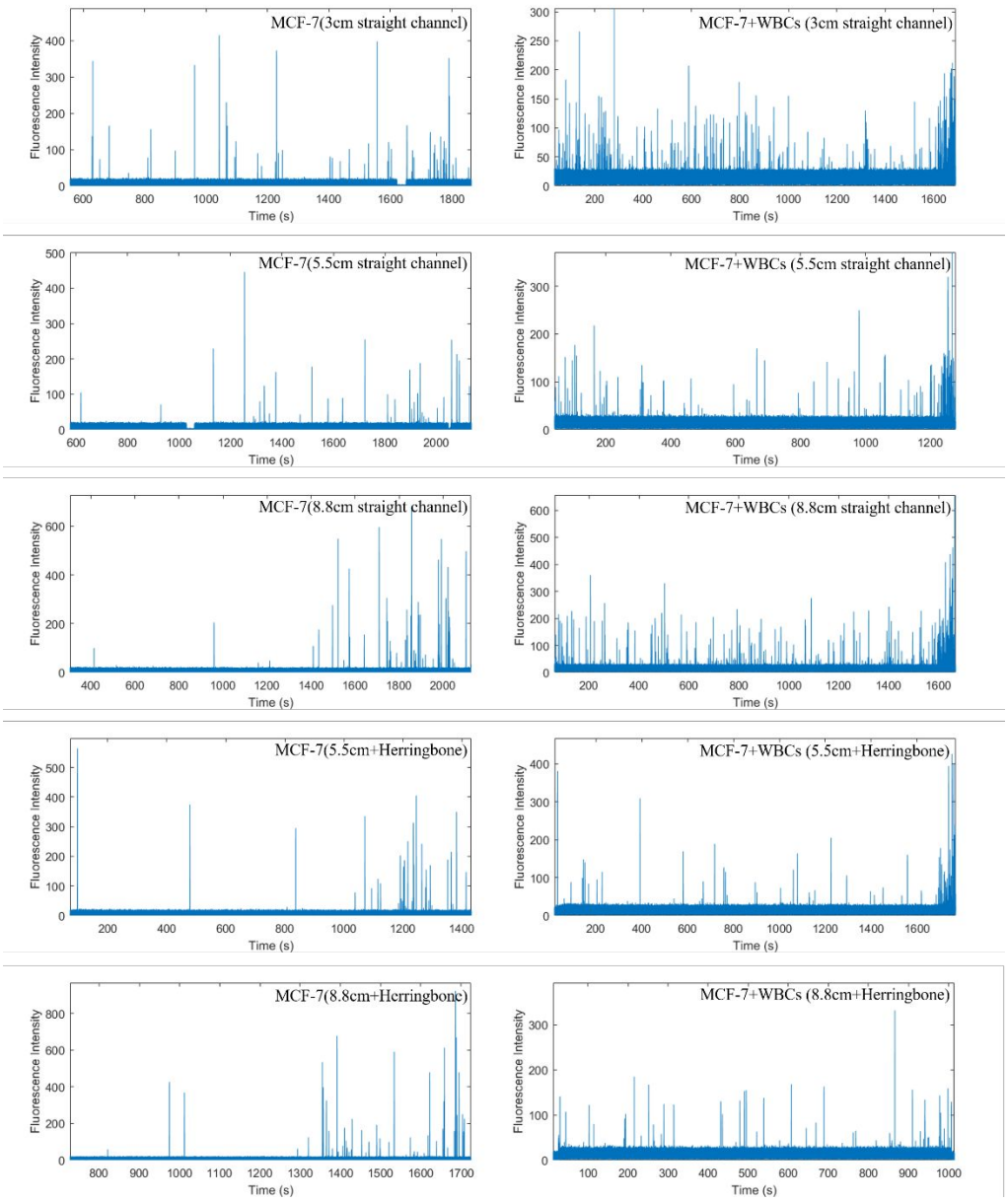

Figure S4. Counting and quantifying the number of MCF-7 cells and total nucleated white blood cells (MCF-7+WBCs) collected from five chips with different flow-stretching channels: 3cm straight channel, 5.5cm straight channel, 8.8cm straight channel, 5.5cm+Herringbone and 8.8cm+Herringbone. Panels on the left show the complete trace records for MCF-7 cells (labeled with PE tagged anti-EpCAM). Panels on the right show the complete trace records for MCF-7 cells plus WBCs (labeled with PE tagged anti-EpCAM and anti-CD45).

Table S1. Tabulation of the number of spiked-in MCF-7 cells and the WBCs that were recovered from five sequential eDAR chips of different flow-stretching designs.

	3cm	5.5cm	8.8cm	5.5cm+HB	8.8cm+HB
MCF-7	54	29	57	30	49
MCF-7+WBCs	313	141	177	100	70
Purity	17.3%	20.6%	32.2%	30.0%	70.0%