Electronic supplementary information (ESI) for

Double Spiral Microchannel for Label-free Tumor Cell Separation and Enrichment

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Construction of confocal microscopic images

In this experiment, we first mounted the double spiral device onto the stage of a Zeiss LSM 710 confocal microscope, focused the S-turn region (or trifurcated outlet) of the double spiral channel, and split the z-axis into fifteen slices (the interval between two slices was 50/14=3.57 μm). Then, we injected the 5- or 15-μm-diameter particles into the double spiral channel through a syringe pump at the flow rate of 20 mL/hr, waited 20 s for particles reaching equilibrium states, and started to capture slices. The confocal microscope took approximately 4 s to scan one slice, and the total time required for scanning 50 μm deep channel was around 1 min. During image acquisition, the fluid containing particles was continuously flowing through the double spiral channel at 20 mL/hr to keep the steady states of particles. After obtaining scanned slices, we used Zen 2009 Zeiss software suite (Carl Zeiss) to automatically construct the 3-D images showing the particle trajectories (Fig. S5).
Fig. S1 Simulation prediction of velocity field at the S-shape junction under 20 mL/hr showing a reversal in the direction of the Dean vortex for consecutive anticlockwise and clockwise spirals. The vector arrows are not drawn proportionally to the velocity magnitude and the velocity magnitude is denoted by contour colors.
**Fig. S2** Numerical simulation of trajectories of two types of particles at (a) the inlet, and (b) S-turn to show focusing process at 20 mL/hr. 5 μm particles are indicated by red dots, and 15 μm particles are indicated by blue dots.
**Fig. S3** (a) Fluorescent trajectories of (a) 5 μm particles (red stream), and (b) 15 μm particles (green stream) along the 6-loop double spiral channel at the flow rate of 20 mL/hr. The particles first migrate toward the center of double spiral in the counterclockwise direction from Loop 1 to 6, pass the S-shape junction and migrate out in the clockwise direction from Loop 6' to 1'. 1 represents the outermost loop in the counterclockwise direction through which originally randomly distributed 5 μm particles are introduced into the double spiral channel. 1' represents the outermost loop in the clockwise direction from which the focused 5 μm particles flow out. (c) Composite fluorescent image showing the position and distribution of 5 μm (red stream) and 15 μm (green stream) particles along the double spiral channel.
**Fig. S4** The mean position of 5 μm particles (red) and 15 μm particles (green) from the inner channel wall in each loop of the double spiral channel at the flow rate of 20 mL/hr. The error bar indicates the width of particle stream.
**Fig. S5** Confocal fluorescent microscopic images revealing the 3-D distribution of 5 μm (red) and 15 μm (green) particles at (a) the center, and (b) the outlet of the double spiral channel at 20 mL/hr. The dash white line represents the channel boundary.
Fig. S6 The mean position of diluted blood (blue), Hela cells (green) and MCF-7 cells (red) from the inner channel wall in each loop of the double spiral channel at the flow rate of 20 mL/hr. The error bar indicates the width of cell stream.