Supporting Information

Deterministic Sequential Isolation of Floating Cancer Cells under Continuous Flow
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Flowing paths of microbeads in a zero-offset microfluidic device

We have performed a preliminary experiment by flowing microbeads into a testing ‘enlarged’ microfluidic device containing micro-sieves locate perfectly along the axial center of the microchannel without initial offset and sieve offset. We observed that after the first bead was trapped at the first micro-sieve, all the following beads detoured through either the positive-y or negative-y side of the microchannel as shown in Figure S1. The downstream flowing path of each the detoured bead would have an offset of ~35 µm from the channel center, in either the positive-y or negative-y direction depending on which side the bead bypassing the first micro-sieve. Apparently, such offset was caused by the physical contact between the bead and the first micro-sieve. Indeed, we have considered this physical offset as our first reference for the trapper offset values as mentioned in the main-text.

Isolation of micro-beads using a ‘true-scale’ microdevice

Based on the design of enlarged microdevice, dimensions of the device scaled down isometrically with a ratio of 4:1 as the ‘true-scale’ to match the size of cancer cells. The corresponding scaled Reynolds number was very small and therefore the flow streamlines were identical to the enlarged device. To verify the sequential isolation capability, microbeads with the diameter of 20 µm (density: 5 × 10^3 bead mL^-1) were injected into the true-scale device with the sample flow rate of 2 µL min^-1 and buffer flow rate of 40 µL min^-1. Results (Figure S2) show that the isolation performance was comparable to that of the enlarged devices.
**Figure S1.** (a) The first bead is trapped at the first micro-sieve. (b) The subsequent bead detours around the positive-\(y\) side of the first trapper and exits the microchannel. (c) Another incoming bead detours around the negative-\(y\) side of the first trapper. Scale bar: 200 \(\mu\)m.

**Figure S2.** Sequential isolation of microbeads with a 20 \(\mu\)m diameter in micro-sieves (top to bottom) using a 'true-scale' microdevice. Scale bar: 100 \(\mu\)m.