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.

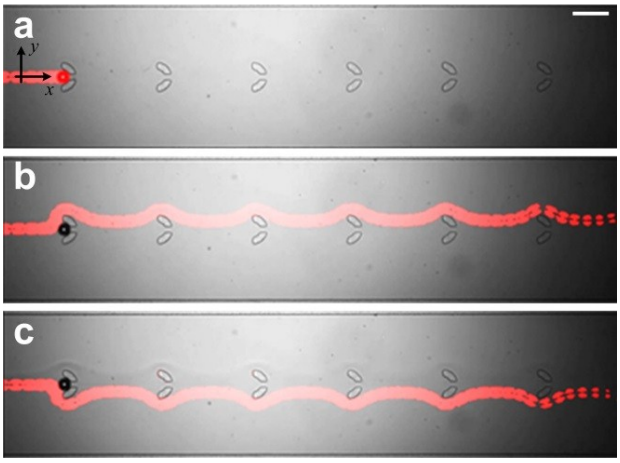

Figure S1. (**a**) The first bead is trapped at the first micro-sieve. (**b**) The subsequent bead detours around the positive-*y* side of the first trapper and exits the microchannel. (**c**) Another incoming bead detours around the negative-*y* side of the first trapper. Scale bar: 200 μm.

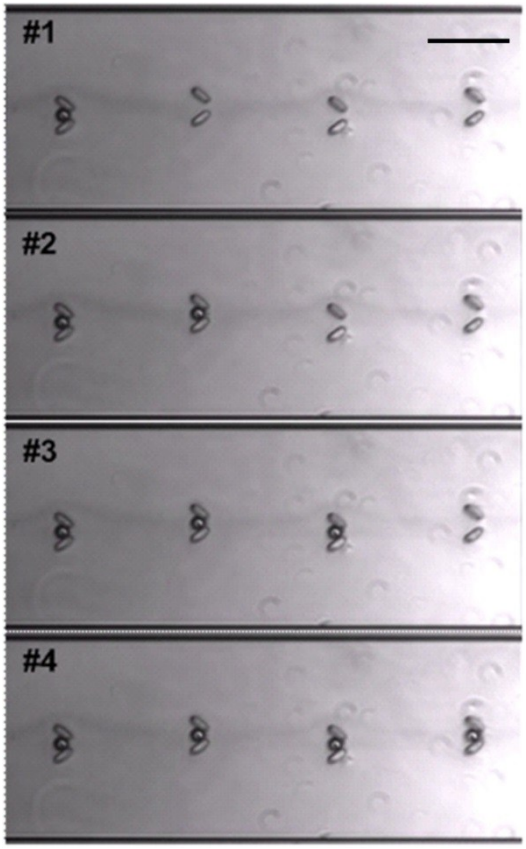

Figure S2. Sequential isolation of microbeads with a 20 μm diameter in micro-sieves (*top* to *bottom*) using a 'true-scale' microdevice. Scale bar: 100 μm.