Supporting information:

Ensemble of Aptamers and Antibodies for Multivalent Capture of Cancer Cells

1. Tumor cells.

T-cell human acute lymphoblastic leukemia cells (CCRF-CEM cells, CCL-119) and B-cell human Burkitt's lymphoma cells (Ramos cells, CRL-1596) were purchased from American Type Culture Collection (ATCC). Antigen expression on the cell surface was confirmed by flow cytometry. All cells used in our experiments were maintained in incubators at 37 °C with 5% CO_2. CEM and Ramos cells were grown in RPMI 1640 medium (ATCC) supplemented with 100-units/mL antibiotic-antifungal solutions of penicillin-streptomycin (Cellgro, Manassas, VA, USA) and 10% fetal bovine serum (FBS; heat-inactivated; Gibco). Just before use, cells were washed three times with 2 mL Dulbecco's phosphate-buffered saline with calcium and magnesium ions (PBS) (Fisher Scientific, Pittsburgh, PA, USA). Cell concentrations were determined using a hemacytometer before each experiment. Specific cell concentrations are detailed in the figure captions. A solution of 1 % (w/v) bovine serum albumin (BSA) (Fisher) and 0.05% (w/v) Tween-20 (Fisher) in PBS was used for re-suspending cells for cell capture.

2. Affinity surface preparation.

First, the channels were rinsed with 300 µL of ethanol at a flow rate of 2 µL/s using a Micro4 syringe pump (World Precision Instruments, Sarasota, FL, USA) with a 1 mL syringe connecting to the inlet of the device using polymer tubing. Secondly, the device was washed with 300 µL PBS buffer at 2 µL/s. Then, 100 µL of avidin (Invitrogen, Carlsbad, CA, USA) with a concentration of 1 mg/mL was dispensed at the inlet. It was introduced into the device by applying vacuum to the outlet of the device with very low pressure and the incubation was allowed for 15 min at room temperature. Avidin was immobilized on the channel surface by physical adsorption. Next, a PBS buffer solution containing biotinylated anti-PTK7 (Miltenyi Biotec, Auburn, CA, USA) and biotinylated sgc8 aptamers at a predetermined ratio, 1% (w/v) bovine serum albumin BSA, and 0.05% (w/v) Tween-20 was introduced into the device. The solution was incubated in the channels for 15 min at room temperature. The device was then rinsed with 300 µL of PBS buffer containing 1% (w/v) BSA and 0.05% (w/v) Tween-20. At room temperature, through biotin-avidin interaction, the capture agents (sgc8 aptamers and anti-PTK7) were immobilized onto the substrates, enabling a highly efficient cell capture at a high flow rate due to the size-different effect of binding reagents.

The sgc8 aptamers were prepared using our previously reported method.[1] Flow cytometry was used to verify their specific bindings with the targeted CEM cells (Figure 1S).

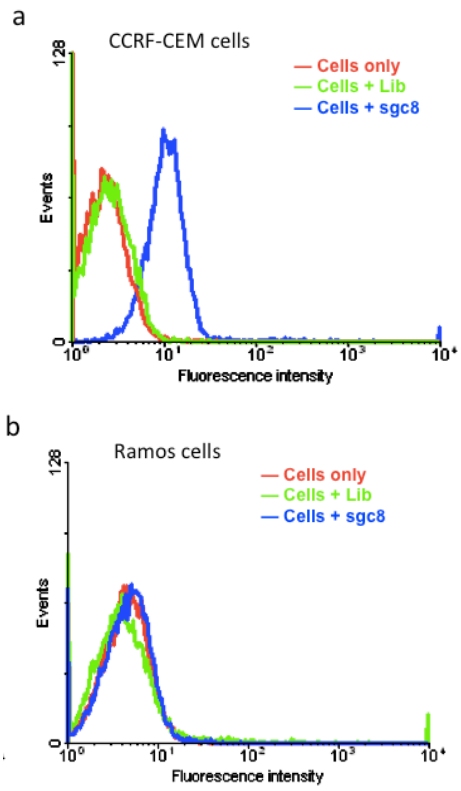

Figure 1S. Flow cytometer testing of the specific binding of CCRF-CEM cells and sgc8 aptamers. (a) CEM cells selectively bind with sgc8 aptamers; negligible signal change was observed for cells incubated with random DNA library (Lib) compared with cells only. (b) Control Ramos cells did not have selective binding with sgc8.

Different mixtures of antibodies and aptamers were used for verifying the ensemble. They are listed in Table 1S.

(Anti-PTK7 to sgc8 ratio)	Anti-PTK7 (µg/mL)	sgc8 (µM)
1:30	5	1
1:300	5	10
1:3000	0.5	10

Table 1S. Mixtures of antibodies and aptamers used for verifying the antibody-aptamer ensemble.

3. Cell capture using aptamer and antibody ensemble in a microfluidic device.

To carry out the cell capture experiments, cells were washed with a PBS buffer immediately before the experiment, and then resuspended at a concentration of 10^6 cells/mL. CEM and Ramos cells were stained respectively with Vybrant DiO (green) and DiI (red) cell-labelling solutions (Invitrogen,

Carlsbad, CA, USA). Then the cells were washed with PBS, and resuspended in PBS containing BSA and Tween-20. Flow control in this experiment was achieved using syringe pumps. The syringe holding the cell suspension was connected to the device using polymer tubing.

To start the cell capture experiments, the device was first immobilized with an ensemble of sgc8 aptamer and anti-PTK7 antibody on the surface of microchannels, followed by three rinses with the PBS containing 1% BSA and 0.05% Tween-20. Then, 1 mL of DiO-stained CEM and DiI-labeled Ramos cell suspension in PBS buffer was continuously pumped into the device. After the device was washed three times with PBS to remove nonspecifically bound cells, fluorescent images were acquired to determine the cell numbers. The device was placed on the stage of an Olympus IX71 fluorescence microscope for image acquisition. Different sets of images corresponding to the green fluorescent cells, red fluorescent cells, and transmission images were acquired at each position inside channels to determine cell numbers. Software Image Pro Plus (IPP) was used for cell counting. Cell counts were further confirmed by comparing fluorescent images with transmission images to get those real target cells with appropriate cell morphology in the transmission images. The cell capture efficiency was calculated by dividing the number of the target cells captured by the number of total target cells introduced into the device, while the cell purity was determined by dividing the number of the target cells captured by the number of the total cells captured, which included both target cells and the unspecific bound cells.

To enable the efficient capture of CTCs from whole blood, we introduced anti-PTK7 antibody and sgc8 aptamer ensemble into a micropillar-based microfluidic device. A series of artificial CTC blood samples were prepared by spiking different concentrations of CEM cells into human blood (Novi, MI, USA), containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). To test the ensemble-capture for blood samples, CEM cells were pre-stained with DiI in red color, and then spiked in whole blood. To verify the purity of captured cells from whole blood, DAPI (4,6-diamidino-2-phenylindole, Invitrogen) was introduced into the device to label the nonspecifically captured white blood cells after the cell capture experiment and rinsing. For whole blood samples, the target CEM cells were positive to both DAPI and DiI (blue merged with red), while cells positive to DAPI only were white blood cells (blue only).

4. Flow condition optimization.

Flow rates ranging from 1.0 μL/s to 3.0 μL/s of the system using antibody and aptamer ensemble were investigated and their effects on the cell capture purity were showed in Figure 2S.

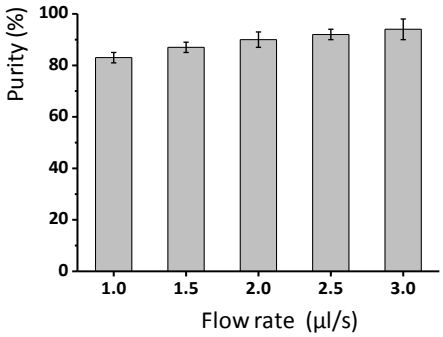

Figure 2S. Capture purity of target CEM cells at a flow rates ranging from 1.0 to 3.0 μL/s with antibody-aptamer ensemble; Error bars represent one standard deviation (n = 3).

5. Cells capture comparison between antibody-aptamer ensemble and aptamer or antibody alone.

To examine the effect of the ensemble on cell capture, we first compared the capture efficiency of the ensemble-capture with antibody-alone-capture at a flow rate of 1.2 μL/s. Results showed that both the capture efficiency and purity of the ensemble-capture is higher than the antibody-alone-capture (Figure 3S)**.**

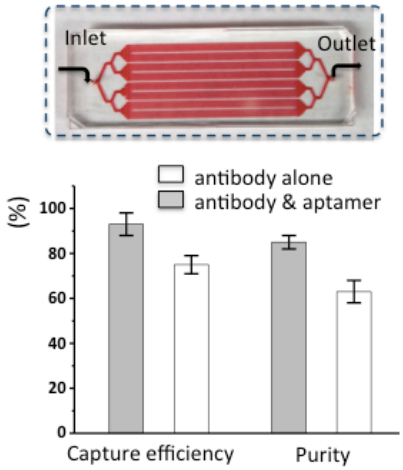

Figure 3S. Comparison of captures efficiency and cell purity between the ensemble-capture and antibody- alone-capture at a flow rate of 1.2 μL/s. Insert is a picture of the device.

To capture CEM cells using antibodies alone, 5 μg/mL biotinylated anti-PTK7 was introduced into the device to immobilize antibodies on the channel surfaces at a flow rate of 2.0 μL/s. CEM cell samples in PBS buffer ranging from 10^2/mL to 10^5/mL were used for cell capture. The calibration curve is shown in Figure 4S. The capture efficiency is (60 ± 4)%.

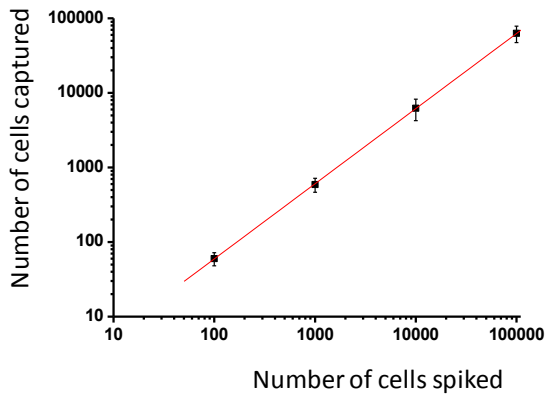

Figure 4S. Calibration curve obtained by using anti-PTK7 alone for 100 000, 10 000, 1000, and 100 CEM cells spiked in 1 mL PBS buffer with a flow rate of 2.0 µL/s.

The same cell capturing tests were performed using 20 µM sgc8 aptamer alone, with a capture efficiency of (58 ± 6)%. The calibration curve is shown in Figure 5S.

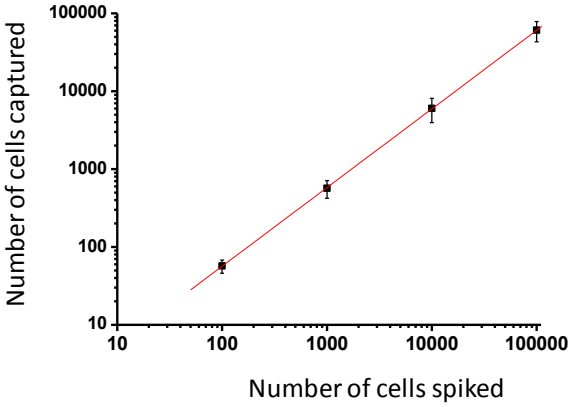

Figure 5S. Calibration curve obtained by using sgc8 aptamer alone for 100 000, 10 000, 1000, and 100 CEM cells spiked in 1 mL PBS buffer with a flow rate of 2.0 µL/s.

6. Microfluidic devices.

The eight channels were connected to form a high throughput device inspired by several works in the literature.[2-4] We designed each channel with a length of 50 mm, a width of 2.1 mm, and a depth of 100 µm. The distance between the micropillars is 60 µm and each pillar's diameter is 90 µm. The devices were made of polydimethylsiloxane (PDMS) and bonded to a glass slide. PDMS devices were fabricated using soft lithography.[5] The layout of the device was first designed in AutoCAD, and a high-resolution transparency photomask was printed. The master used to create the fluidic channels was made by spin coating a layer of SU-8 2035 photoresist (MicroChem, Newton, MA, USA) on silicon wafers (Silicon Inc., Boise, ID, USA) using a spin coater (Laurell Tech., North Wales, PA, USA). Followed by UV exposure and development, a silicon master patterned with the complementary structures of the photomask was created. The channel depth was controlled by the spin speed of SU-8, and measured with a Dektak 150 profilometer. PDMS layer was obtained by casting a liquid PDMS precursor (Dow Corning, Midland, MI, USA) against the silicon master. The PDMS layer was cured in an 80ºC oven for 50 minutes. It was later removed from the master and snatch edges were removed. The inlet and outlet holes were introduced onto the PDMS layer at the end of the channel. The PDMS layer was then sealed with a glass slide (3 in. × 1 in.), and the fluidic channel was generated. The PDMS chip could be reused after cleaning with a suitable solvent.

References:

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