Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic supplementary information

Rapid separation of human breast cancer cells from blood using a simple spiral channel device

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Table S1. Concentration and volume of blood cells and MCF-7 cells collected from each outlet after first stage separation. The initial concentrations of diluted blood cells and MCF-7 were about 5×10^7 cells/mL and 5×10^4 cells/mL, respectively.

Outlet	Cell Counter Channel	Measured concentration (cells/mL)			Average Concentration (cells/mL)	Volume (mL)	Cell number
		1	2	3			
Inner	Bright field	1.28×10^8	1.675×10^8	1.505×10^8	1.49×10^8	2.6mL	3.87×10^8
Middle	Bright field	1.16×10^7	1.295×10^7	1.225×10^7	1.23×10^7	2.8mL	3.57×10^7
Middle	Fluorescence	1.11×10^5	1.14×10^5	1.35×10^5	1.20×10^5	2.8mL	3.36×10^5
Outer	Bright field	1.135×10^6	1.19×10^6	1.16×10^6	1.16×10^6	2.5mL	2.90×10^6

In this table, we can count the total cell numbers through the bright field channel and the tumor cell numbers through the fluorescence channel. The efficiency of separation are calculated as follows:

(1) In the inner and outer outlets, since the number of MCF-7 cells are several orders of magnitude smaller than the background cells, we can assume that the numbers of total cells are the numbers of blood cells. In the middle outlet, the number of blood cells is 3.57×10^7-3.36×10^5=3.54×10^7;

(2) The percentage of blood cells removed from inner and outer outlets after first stage separation: $(3.87\times10^8+2.90\times10^6)/(3.87\times10^8+3.54\times10^7+2.90\times10^6)$=91.68%;

(3) The recovery rate of MCF-7 cells: $(3.36\times10^5)/(5\times10^4/\text{mL}\times7.9\text{mL})$=85.06%;

(4) The purity of the collected MCF-7 cells: $(3.36\times10^5)/(3.57\times10^7)$=0.94%.

Table S2. Concentration and volume of blood cells and MCF-7 cells collected from each outlet after second stage separation.

Outlet	Cell Counter Channel	Measured concentration (cells/mL)			Average Concentration (cells/mL)	Volume (mL)	Cell number
		1	2	3			
Inner	Bright field	3.685×10^7	3.095×10^7	3.335×10^7	3.37×10^7	0.6mL	2.02×10^7
Middle	Bright field	3.33×10^6	3.125×10^6	3.18×10^6	3.21×10^6	0.9mL	2.89×10^6
Middle	Fluorescence	3.66×10^5	1.91×10^5	2.23×10^5	2.60×10^5	0.9mL	2.34×10^5
Outer	Bright field	2.465×10^5	1.995×10^5	2.55×10^5	2.34×10^5	0.7mL	1.64×10^5

As the same, we can count the total cell numbers through the bright field channel and the tumor cell numbers through the fluorescence channel through this table. The efficiency of separation are calculated as follows:

(1) In the inner and outer outlets, since the number of MCF-7 cells are several orders of magnitude smaller than the background cells, we can assume that the numbers of total cells are the numbers of blood cells. In the middle outlet, the number of blood cells is 2.89×10^6-2.34×10^5=2.66×10^6;

(2) The percentage of blood cells removed from inner and outer outlets in the second stage separation: $(2.02\times10^7+1.64\times10^5)/(2.02\times10^7+2.66\times10^6+1.64\times10^5)$=88.45%;

(3) Ignore the sample loss, the percentage of blood cells removed after first and second stage separation: 91.68%+(1-91.68%)×88.45%=99.04%;

(4) Ignore the sample loss, the recovery rate of MCF-7 cells after first and second stage separation: $85.06\%\times(2.34\times10^5/2.2\times2.8)/(3.3^6\times105)$ =75.40%;

(5) The purity of the collected MCF-7 cells after two stage separation: $(2.34\times10^5)/(2.89\times10^6)$=8.1%;

(6) The enrichment factor of MCF-7 cells at the middle outlet after two stage separation: 8.1%/0.1%=81.

Table S3. Concentration and volume of WBCs and MCF-7 cells collected from each outlet after separation. The initial concentrations of lysed buffy coat and MCF-7 were about 5×10^6 cells/mL and 5×10^4 cells/mL, respectively.

Outlet	Cell Counter Channel	Measured concentration (cells/mL)			Average Concentration (cells/mL)	Volume (mL)	Cell number
		1	2	3			
Inner	Bright field	0.975×10^7	1.005×10^7	1.05×10^7	1.01×10^7	1.4mL	1.41×10^7
Inner	Fluorescence	2.35×10^4	1.76×10^4	1.47×10^4	1.86×10^4	1.4mL	2.60×10^4
Middle	Bright field	3.625×10^6	3.98×10^6	4.235×10^6	3.95×10^6	1.5mL	5.93×10^6
Middle	Fluorescence	1.29×10^5	1.32×10^5	1.41×10^5	1.34×10^5	1.5mL	2.01×10^5
Outer	Bright field	2.26×10^5	3.14×10^5	2.755×10^5	2.72×10^5	1.5mL	4.08×10^5
Outer	Fluorescence	0	2.93×10^3	0	0.98×10^3	1.5mL	1.47×10^3

In this table, we can also count the total cell numbers through the bright field channel and the tumor cell numbers through the fluorescence channel. The efficiency of separation are calculated as follows:

(1) In the inner outlet, since the number of MCF-7 cells are three orders of magnitude smaller than the WBCs, we can assume that the numbers of total cells are the numbers of WBCs. In the middle and outer outlets, the number of WBCs are 5.93×10^6-2.01×10^5=2.73×10^6 and 4.08×10^5-

$1.47\times10^3=4.07\times10^5$, respectively.

(2) The percentage of WBCs removed from inner and outer outlets after separation: $(1.41\times10^7+4.07\times10^5)/(1.41\times10^7+5.73\times10^6+4.07\times10^5)=71.69\%$;

(3) The recovery rate of MCF-7: $2.01\times10^5/(2.60\times10^4+2.01\times10^5+1.47\times10^3)=87.98\%$;

(4) The purity of the collected MCF-7 cells: $(2.01\times10^5)/(5.93\times10^6)=3.39\%$;

(5) The enrichment factor of MCF-7 cells: 3.39%/1%=3.39.

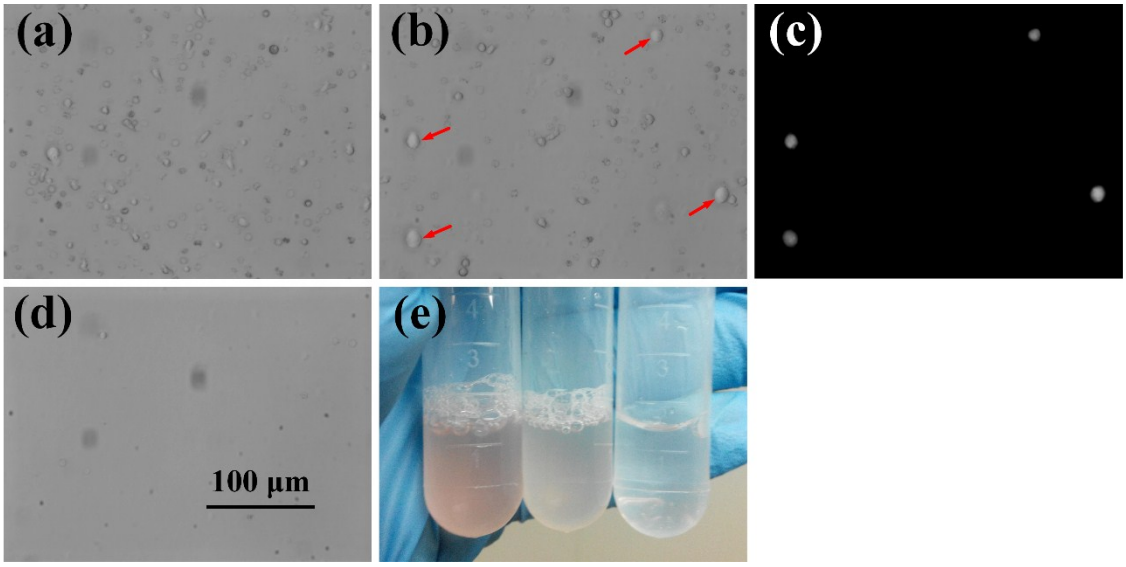

Fig. S1. The separation of WBCs and MCF-7 cells whose initial concentrations are about 5×10^6 cells/mL and 5×10^4 cells/mL respectively. The Figure is combined with the microscopic images of samples collected from (a) inner outlet under bright field, (b) middle outlet under bright field, (c) middle outlet in fluorescence mode and (d) outer outlet. (e) Photographs of samples collected from each outlet.

Table S4. Concentration and volume of WBCs and MCF-7 cells collected from each outlet after separation. The initial concentrations of lysed blood cells and MCF-7 were about 5×10^6 cells/mL and 500 cells/mL, respectively.

Outlet	Cell Counter Channel	Measured concentration (cells/mL)			Average Concentration (cells/mL)	Volume (mL)	Cell number
		1	2	3			
Inner	Bright field	1.04×10^7	1.125×10^7	1.18×10^7	1.12×10^7	1.0mL	1.12×10^7
Inner	Fluorescence	1.47×10^4	1.17×10^4	2.64×10^4	1.76×10^4	13μL	228.8
Middle	Bright field	3.745×10^6	3.935×10^6	4.04×10^6	3.91×10^6	1.3mL	5.08×10^6
Middle	Fluorescence	3.05×10^5	2.43×10^5	1.06×10^5	2.18×10^5	10μL	2180
Outer	Bright field	1.495×10^5	1.145×10^5	1.32×10^5	1.32×10^5	1.2mL	1.58×10^5
Outer	Fluorescence	0	0	0	0	10μL	0

In this experiment, since the number of MCF-7 cells were extremely low in the collected samples while the concentration can be counted by the Cell Counter is at least 1×10^4 cells/mL, we have to concentrate the collected samples to a very small volume by the centrifugation. This may lead to a relatively large measurement error. However, we can still capture the most of tumor cells from the middle outlet (see Fig S2 (d) and (e)) in the case that there are very rare tumor cells in the initial sample. The result shows that our device have a great potential for biomedical and clinical applications.

By the way, the percentage of WBCs removed from inner and outer outlets after separation is $(1.12\times10^7+1.58\times10^5)/(1.12\times10^7+5.08\times10^6+1.58\times10^5)=69.10\%$, which is close to 71.69% calculated from the separation experiments with larger concentrations of MCF-7 cells.

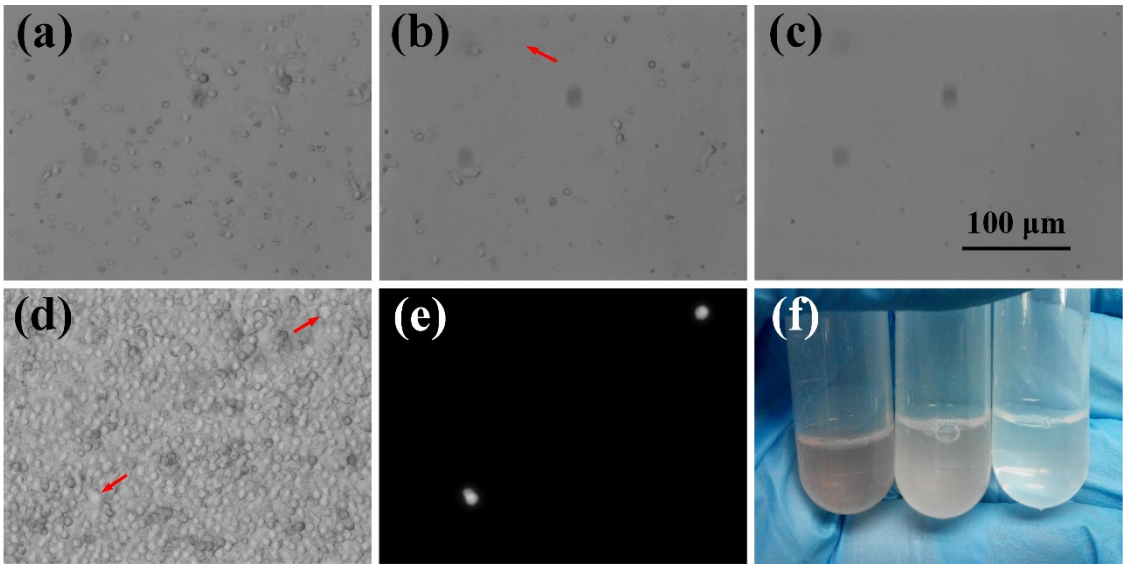

Fig. S2. The separation of WBCs and MCF-7 cells whose concentrations were 5×10^6 cells/mL and 500 cells/mL respectively. The Figure is combined with microscopic images of samples collected from (a) inner outlet, (b) middle outlet and (c) middle outlet under bright field. To determine the separation performance, sample collected from middle outlet was concentrated by centrifugation to 10 µL. Images taken (d) under bright field and (e) in fluorescence mode show that the stained tumors cells still can be detected. (f) Photographs of samples collected from each outlet.