New generation of ensemble-decision aliquot ranking based on simplified microfluidic components for large-capacity trapping of circulating tumor cells

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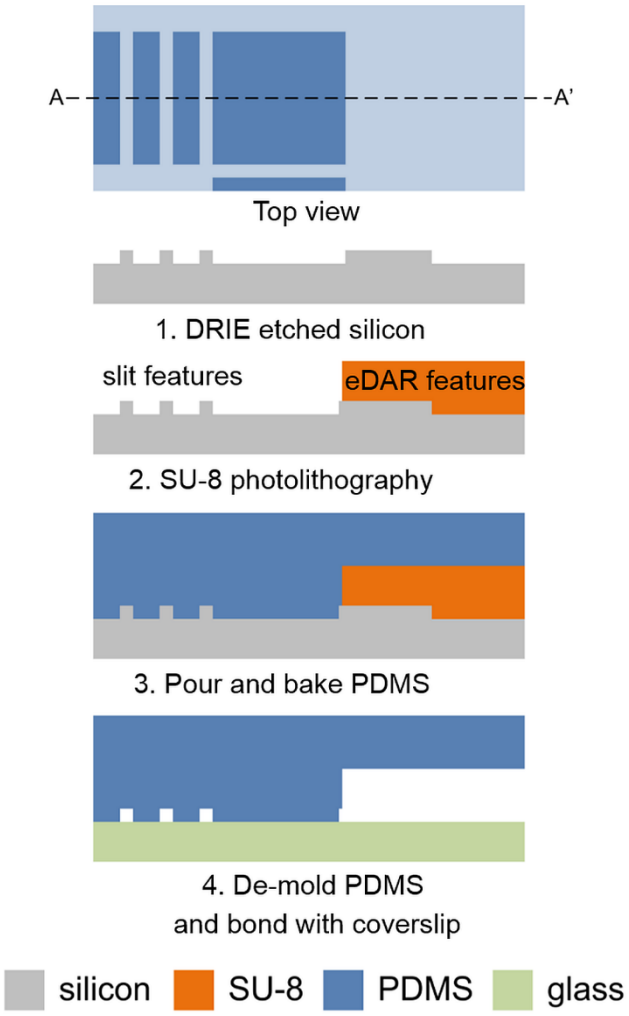

Figure 1S The process flow for the microfabrication steps. The microfeatures for the slit filters were fabricated using positive resist lithography and deep reactive ion etching (DRIE) process. The second layer of the eDAR chip was fabricated using SU-8-3050 as a negative photoresist.

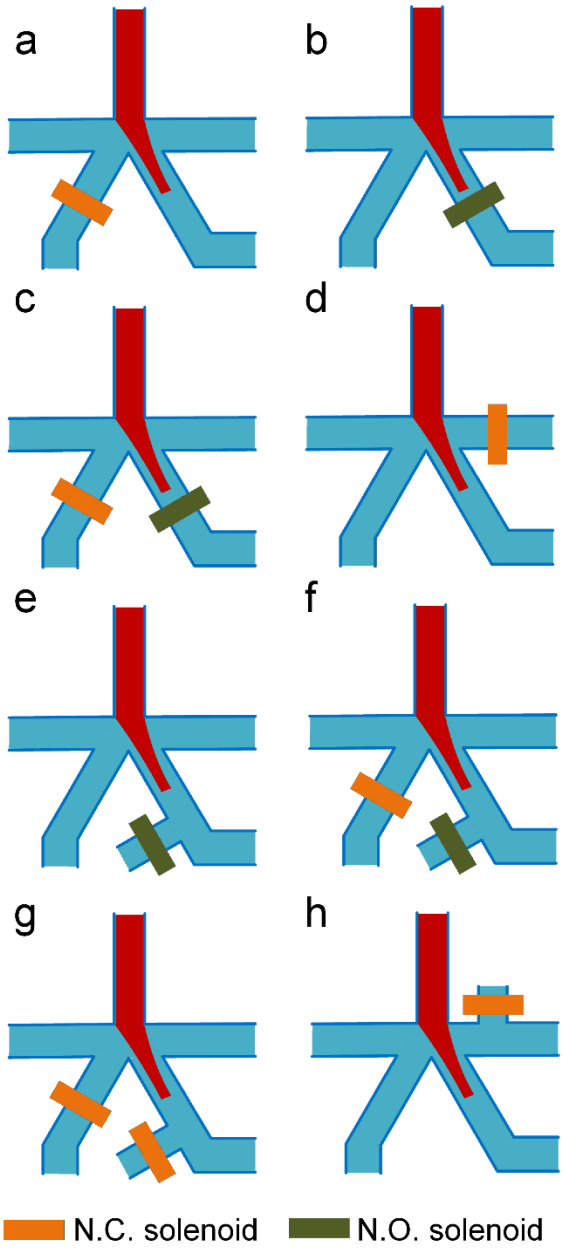

Figure 2S Eight hydrodynamic sorting schemes. The blood was injected from the main channel, shown as the red flow. Buffer (Blue) flowed in the two side channels, CTCs were collected to the bottom left channel, and the waste was directed to the bottom right channel. The rectangular blocks represents the solenoid. If the solenoid was set to be normally open (N.O.), the color of the block is set to green; if the solenoid was set to be normally closed (N.C.), the color is set to orange. See table 1S for more information.

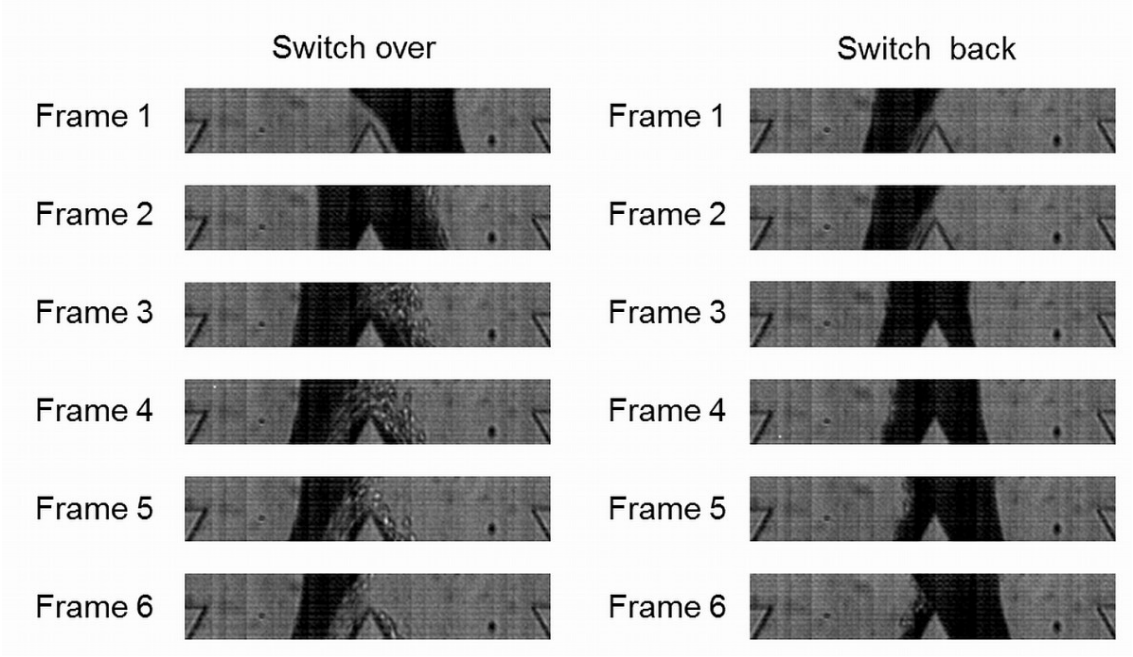

Figure 3S Switching time for the current fluidic scheme recorded by high speed camera. The frame rate was 1918 fps, so the average switch over time was about 2 ms, and the switch back time was also 2 to 3 ms.

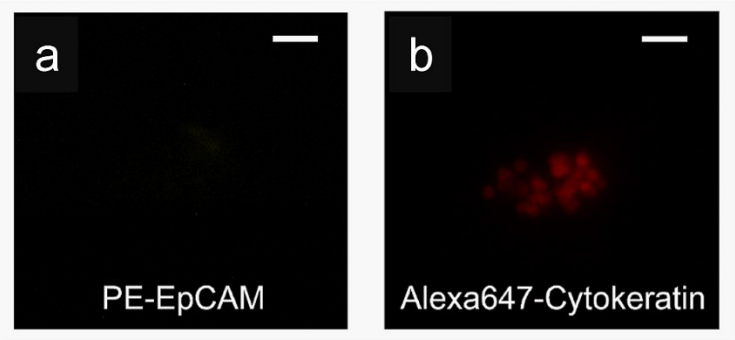

Figure 4S A CTC cluster with low EpCAM expression from a pancreatic cancer sample (No. 20). The scale bar is 100 μm.

Scheme	Position	Normal state	Left pressure (psi)	Right pressure (psi)	Switch over time (ms)	Switch back time (ms)
a	Collection	Closed	Low	High	~2-3	~15-25
b	Waste	Open	High	Low	~15-20	~2-3
c	Collection	Closed	Low	High	~4-5	~10
	Waste	Open				
d	Right Buffer	Close	Low	High	~3	~40
e	Waste	Open	High	Low	~25	~2
f	Collection	Closed	Low	High	~25	~5-6
	Center Waste	Open				
g	Collection	Closed	Low	High	~2-3	~2-3
	Center Waste	Closed				
h	Center Right Buffer	Closed	Low	High	~2-3	~2-3

Table 1S Summary of the fluidic configuration and performance of the 8 sorting schemes we tested. Two solenoids were used in schemes c, e, and g. When there were two outlets or inlets on a single channel, the position of the solenoid was marked. For example in scheme f, the position of the second solenoid was "center waste", meaning that it was placed on the center outlet of the waste collection channel. In every scheme, except g, when the "positive" events were detected, the DC voltage applied on the solenoids immediately was changed to trigger the sorting, and after a certain period of time was changed back to the normal state. Scheme g utilized 4 individual steps to control the sorting. Initially, both solenoids were set to closed, and

the blood flowed to the waste channel. When the sorting was triggered, only the solenoid on the collection side was opened to perform the switch-over step; after the cell was collected, the other solenoid was opened to perform the switchback step. After the blood flow was completely switched back, both solenoids were closed at the same time, same as the normal state.

Sample	Volume (mL)	CTCs counts	Sample	Volume (mL)	CTCs counts	Sample	Volume (mL)	CTCs counts
Control 1	1	0	Control 15	1	0	Patient 14	1	8
Control 2	1	0	Patient 1	1	183	Patient 15	1	2
Control 3	1	0	Patient 2	1	9	Patient 16	1	10
Control 4	1	0	Patient 3	1	7	Patient 17	1	872
Control 5	1	0	Patient 4	1	3	Patient 18	1	2
Control 6	1	0	Patient 5	1	14	Patient 19	1	5
Control 7	1	0	Patient 6	1	6	Patient 20	1	12
Control 8	1	0	Patient 7	1	4	Patient 21	1	22
Control 9	1	0	Patient 8	1	0	Patient 22	1	2
Control 10	1	0	Patient 9	1	0	Patient 23	1	0
Control 11	1	0	Patient 10	1	27	Patient 24	1	14
Control 12	1	0	Patient 11	1	44	Patient 25	1	0
Control 13	1	0	Patient 12	1	5	Patient 26	1	7
Control 14	1	0	Patient 13	1	7			

Table 2S Raw data of the control (n=15) and the pancreatic cancer samples (n=26). Patient sample #1 to #16 were analyzed using the first generation of eDAR; patient sample #17 to #26 were analyzed using the newer generation of eDAR.