Electronic Supplementary Material (ESI) for Analyst.
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Supplementary information

1. The fabrication details of single cell sorting and analysis microfluidic chip

Two separate patterns for microchannels and microwells were designed using Corel DRAW X5 and printed on a transparency film. Firstly, a clean silicone wafer was pre-baked for 30 min at 280℃ and cool to temperature. SU8-2025 (MicroChem, USA) was poured onto the wafer and put into spin coater (Special Coating System, USA) with a program setting of spinning at 500 rpm for 10 s and 2000 rpm for 30 s to form channel layer of approximately 40 μm. Then the wafer was heated up to 95℃ to solidify the photoresist and cool down to temperature and exposed for 20 s under ultraviolet exposure machine (ABM, USA). Subsequently, SU8-2075 (MicroChem, USA) was used to construct microchamber layer of 240 μm with a setting of spinning at 500 rpm for 10 s and 1000 rpm for 30 s. After solidification, the coated wafer was exposed under mask aligner (Sichuan Nanguang Vacuum Technology Co., Ltd, China) for 38 s. Then the photoresist was baked, developed and hard-baked until the mold was finished.

After that, The mold was treated with trichloro-(1H,1H,2H,2H-perfluorooctyl) silane (Sigma-Aldrich) for 10 min to facilitate PDMS release from the mold. Firstly, a 300 μm thick microarray layer was fabricated by spinning 6 g 5 : 1 (A:B) PDMS-curing agent mixture under the speed of 500 rpm for 10 s and 1000 rpm for 30 s , and baking at 85℃ for 2 min until the PDMS polymer hardened. Secondly, 1 mL EGC-1720 was added onto the mold, spun at 500 rpm for 3 s, 1000 rpm for 3 s and baked at 85℃ for 1 min to form an antievaporative nano coating layer. Thirdly, 5 g 10:1 PDMS-curing

agent mixture was poured onto the mold and baked for 10 min at 85℃. Then two screws were separately put above the two inlet channel and 15 g 10:1 PDMS-curing agent mixture was poured onto the mold. After baking for 40 min, the PDMS cast onto the mold was peeled off to punch holes for the inlets. Fourthly, 3 g 30:1 PDMS-curing agent mixture was poured onto a clean glass coverslip, which was subsequently spun at 500 rpm for 10 s and 1000 rpm for 30 s. Then the glass coverslip was heated at 85℃ for 20 min and removed from the heater immediately to bond with the PDMS chip. After baking at 85℃ for 4 h, the constructed chip can be ready for use.

Table. S1 Velocity statistics in three branching channels

Unit μm/s	Area					
	1	2	3	4	5	6
Velocity mean value	3	2	5	6	11	12
Standard Deviation	1	1	1	1	2	2

Table. S2 Velocity statistics of sibling channels located in three different positions of the chip

Unit μm/s	Area				
	1	2	3	Mean (μm/s)	SD
Velocity mean value	367	353	316	345.3	26.4

Table. S3 Dispersion statistics of polystyrene microspheres in chips

The number of PS microspheres per chamber	The number of loaded PS microspheres				
	900	1300	1800	2000	2300
1	712(79.1%)	997(76.7%)	1453(80.72%)	1518(75.9%)	1623(70.56%)
2	21	37	48	42	64
3	4	11	14	18	37
4	1	5	7	5	10
5	0	1	1	4	6
6	0	2	0	2	5
7	0	0	0	1	4
8	0	0	0	0	0
9	0	0	0	0	0
retention[a]	38	55	71	89	115

a: PS microspheres were partially suspended in the channels due to PDMS viscosity and surface roughness.

Table. S4 Dispersion effect of cells with different input in chips

The number of cells per chamber	The number of loaded cells									
	1000					2000				
	1	2	3	4	5	1	2	3	4	5
0	14	15	14	15	16	7	10	10	10	6
1	5	4	4	4	4	8	7	7	7	7
2	0	0	0	0	0	1	0	2	1	3
3	0	0	0	0	0	1	1	0	0	1
4	0	0	0	0	0	0	0	0	0	0
retention	1	1	2	1	0	3	2	1	2	3
Single cell occupancy[b]	25%	20%	20%	20%	20%	40%	35%	35%	35%	35%
SD	0.022					0.022				

b: Single cell occupancy referred to the ratio between the number of microchambers containing single cell and the total number of microchambers in a field of vision under microscope (20 microchambers in total).

Table. S5 ALCAM gene expression in single A549 cells

Cell number	ALCAM Positive Reactions			Mean value	SD
	Test 1	Test 2	Test 3		
400	13	16	21	16.7	4.0
800	31	42	45	39.3	7.4
1600	94	87	113	98	13.5