

Supplementary material S1

Distribution map of trapped cells in the MBM device using different concentrations of cell suspension (10,000-17,500 cells/mL). 12 tables contain 10 x 20 cells represent the microwells presented in the device. Cells were added into the device from position the right to the left. The cell colored in red represent well with 1 cell. White wells are empty cells.

Cell loading concentration at 10,000 cells/mL

Rep. 1 Total cell trapped number = 418 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A		2	3		1			1	2	1	2	5	2			3	6	5	4	10
B				2		1	1		1		2		1		2	4	5	3	3	5
C	5			1	4	2	5	1		2	1	6		3	3	5	3	5	6	6
D		3	1	2	1			1	2	3	2	4		1	4	5	2	3	8	11
E	2		3	2	2	1	1	4		3		5		2	3	2		2	6	4
F					1			2	1		2	9		3	2	3	3		6	15
G	1	3	2	1	2	5				1			3	6	2	3	2		5	13
H	2			3	2	2			2	2	2		5			5		3		10
I						1		1	2		1		2	4		6				6
J				2			1		3			2		3	3		3	4	3	7

Rep. 2 Total cell trapped number = 525 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A		1	2	2	2		2	1	1			4	1	3	3	4			5	9
B			1	4	1	1		2			3	3	4	1	6	2	3	2	4	13
C					5		2		2	5		2		2	4	7		11	12	2
D		5	1	1		2		3		4	1	4		5		8	2	3	10	8
E	2		2		1	6	2	2	1	3		5		6	2	4	3	3	5	15
F			2	2		2	1		4	1		4	1	4	3	4		7		16
G	2	3	1	1	3	9	2	7	5	3	3	3		7	1	5	2	5	4	12
H	6		4	5			1		1			2	2	4	4	9	3	3	3	9
I				1	2	1	2			1	3	4		3	2	7	5	4	2	4
J		1		2			1		3			2		3	3	5		7	3	6

Rep. 3 Total cell trapped number = 486 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A		1				1	3	1	1		2					6	4	3	7	11
B				2	1	1			1		1	2		6	1	5		4	3	9
C		3		2		2				3	1		2	5		4	5	4	12	11
D		2	1		2		4		2				1		4	7	3	6	7	19
E	5	4	3		8			3		1	2	3	2	6	4	5	3	5	4	12
F	1			2		1	1			2				4		7	4	8	3	8
G	1		1	4	6		1		1			4		3		8	4	9	13	9
H	2						2	4					1		5	5		5	6	9
I				1	2	3			5	2		1			3	4		7	15	5
J						2				1	2	3				6	4	9	5	4

Cell loading concentration at 12,500 cells/mL

Rep.1

Total cell trapped number loading = 701 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A			3	1	3	1	1	2		1	1	1	2	1	2		1	7	4	18
B	1	3	5	3	2	5		1		3	2			4	3	7	4	7		15
C	2		3	1	2	1	2	1	1	3	1	2	1	4	4	1	7	8	15	17
D	1	4	4	1	3	2	1		3	1	1	3	3		7	8	6	10	8	19
E	1	5	1	3	1	5	1	2	1	2	4	2		1	4	9		8	9	10
F		3	6	9		1		3	2	1	2	1	3		5	9	7	12	9	9
G		4	1	2	1	1	2		1	2	1	3	4		6	6	15	8	8	12
H		5	4	3	2	1		1	1	2		2	1	1	5		5	9	17	
I	3		3	1	3		2	4	3	1	3	1	1	3	5	8	14	3	7	15
J	1	5	2	1		3	1	1		1	4		1	2	3	9	5	5	15	

Rep. 2

Total cell trapped number loading = 855 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	1		2	1	4	4	3	1	3	1			5	3	1	4	5	11	19	16
B		1		1		1	1	3	1		3	1	5	15	4		9	17	17	14
C	4	3	1	3	1	3	1	1	1	1				6	6	8	10	14	16	18
D	2	1	1	3		1	3	1	4		1	1		1	8		11	16	15	20
E		4	5	2	1	4	1		3	1	1	5	3	2	9	5	8	17	18	7
F	1	4	3	5		5		4	1	3	2	3	5	5	5	9	18	11	9	11
G	2		1	1		1		1		1	5	1		1	4	10	5	18	8	10
H	1	7	3		5	1	3	5	1	1	1	4			5	5	6	19	9	9
I		3	5	3	4			1	1	1		1	3	5	3		7	8	17	13
J		2	1		1		1	2		3	1	3	3	1	3	8	6	6	10	12

Rep. 3

Total cell trapped number loading = 894 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	4	1			1		1	3	1		1		6	1	5	14	9	10	9	20
B		3	1	1	1		4	1	4		1	5	9	9	6	9		15	9	18
C	6	5	3	1		4	1	2		1	1		1	5		13	7	8	10	9
D	2	1	1	5	6	1	6	3	3	4	5	1	4	3	9	6	14	6	11	10
E		3	2	1	4	5		2	1	4	1	1	5		1	1	16	16	15	15
F	3	1		3	1	1	1	1	5	1				1	6	9	17	15	16	14
G			1	3	3	6	1	1	1	4	6		1	3		14		13	14	9
H		3		2	1	5	6	4		3	1	5	6	3	7	5	15	19	15	15
I	2	1	4	1	1	4	7	3		4		1		6	1	3	8	4	19	20
J		2			4	1	3	1		1			1	7		1	16	9	11	5

Cell loading concentration at 15,000 cells/mL

Rep. 1

Total cell trapped number = 1,114 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	1	1	1	1	1		5	1			1	1	3	1	3	16	3	9	12	15
B		5	5	9	4	6	2	8	4	1	4	4	5	1	9	14	12	10	24	10
C	1	1	1	7	1	3	1	1	1	1		4	1	5	14	13	12	13	14	25
D		1	3	1	1	4	4	6	1	1	9	3	15	12	23	13	13	14	14	23
E	1	1	1	6	1	3	3	7	1	1	9	1	1	3	12	15	9	13	13	29
F		1	2	8	7	3	5	1	4		4	1	1	14	12	13	16	1	4	38
G	1	5	1	3	9	6	9	1	1	1	1		1	15	1	4	15	5	12	5
H	6	8	3	9	5	5	3			1	3	4	1	4	1	7	3	1	3	10
I		6	1	4	1	5	2	3	1		6	7	4	3	3	13	19	5	12	9
J		5	1	1	1	1	1	1	1			1	13	9	1	8	14	2	15	5

Rep. 2

Total cell trapped number = 1,017 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A		5	5	1	1	1			2		1	1	2	5	8	17	15	16	16	19
B		1	2	1	1	4	1	5	6		1		3	1	3	1	1	10	17	25
C	1		3	5	2	2	1	3	2	1	3	3	2	2	1	13	13	13	16	32
D	1	1	3	8	8	1	2	1	5	4	1	3	1	4	8	3	13	12	15	30
E	1	1	6	3	1		1	3	2	7	3	4	5	9	1	18	14	16	10	16
F	2	5	3	2	1	1	1	8	9	2	1	2	1	1	8	9	12	10	13	6
G	5	7	8	1	1	3	2	1	2	1	3	6	7	9	4	1	9	9	18	5
H	3	1	1	2	1	2	1	2	3	1	3	5	2	1	4	1	15	12	18	9
I	1	1	1	2	1	1	8		1	3	1		9	9	5	12	4	13	9	15
J		5			1	5	6	2		2			1	5	6	1	3	14	11	12

Rep. 3

Total cell trapped number = 1,073 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A		6	2	1	1	2	8	2	1		1	1		8	18	7	15	16	15	16
B		1	8	1	1	1		1	1	8	1	1	2	12	13	1	1	17	13	15
C	1	1	4	9	9	3	2	1		1	4	3		3	1	3	3	1	19	12
D	1	1	6	9	1	9	4	1	1	6	1	3	3	1	1	13	13	9	16	30
E	1	1	8	2	1	1	1	4		4	2	1	2	1	3	18	14	16	16	16
F	2	1	5	1		7	1	9	8	1	7	1	14	5	13	13	12	18	16	19
G	7	5	1	1	1		1	3	2		1	4	1	1	1	1	4	23	19	18
H	1	1	6	2		1		1	1	2	2	15	1	9	3	1	1	9	15	12
I	1	1		1	1	1	1	1	3	3	3	2	14		2	12	14	13	8	17
J	1		7	1	9	7		5	1	2		9	8	5		6	13	4	14	14

Cell loading concentration at 17,500 cells/mL

Rep.1

Total cell trapped number = 1,318 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	5	6	5	5	5	4	4	6	6	8	5	9	7	6		17	5	18	6	16
B	6	5	3	7	1	5	6	4	3	9	6	2	8	11		11	4	14	7	15
C	5	3	5	1	1	7	1	1	9	4	1	1	8	2	5	4	3	30	16	20
D	7	5	9	2	1	6	8	8	8	1	6	1	12	14	5	4	13	24	15	25
E	5	1	6	1	1	8	5	5	7	1	1	6	5	1	12	6	4	19	10	26
F	8	5	3	2	4	3	5	7	1	6	7	8	11	4		9	2	14	14	17
G		3	7	3	7	5	4	7	1	4	3	1	5	8	1	3	14	13	15	15
H	8	3	4	5	1	6	5		4	8	4	1	1	11	4	3	5	20	8	14
I	7	4	1	1	5	4	3	2	1	5		7	1	2	9	3	14	15	19	19
J	4	6	4	3	1	1	4	5	1		8	3	1	4	16	5	13	16	16	20

Rep. 2

Total cell trapped number = 1,386 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	5	1	5	7	1		1	4	3	6	1	2	7	4	16	7	15	8	4	20
B	6	7	6	8	4	5	1		5	8	1	4	9	13	3	11	14	6	17	23
C	8	5	3	4	4	4	1	4	1	7	3	3	10	12	1	14	13	20	17	22
D	7	4	4	3	1	5	1	6	5	3	5	15	2	4	1	7	13	14	16	25
E	4	3	7	4	8	3	9	1	1	3	4		10	4	2	8	14	9	12	36
F	7	1	5	1	5	9	1	6	5		2	1	9	1	4	8	23	24	6	27
G	6	1	7	4	4	9	6	9	4	5		5	1	1	3	8	16	23	5	35
H	7	7	1	1	4	8		1	1	4	1	7	9	4	7	9	4	10	18	4
I	8	6	9	5		9	6	1	3	3	3		9	5	19	13	16	5	9	9
J	5	9	8		4	4	3		3	2			6	14	6	15	16	6	26	10

Rep. 3

Total cell trapped number = 1,496 cells

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	1	7	1	10	3	4	5	1	2	5	5	1	1		6	17	5	2	14	10
B	3	2	5	2	1	5	3	4	8	1	10	5	7	3	13	21	12	16	20	34
C	7	2	9	5	3	1	6	5	4	5	5	1	15	12	2	12	16	30	7	12
D	4	3	1	3	1	6	8	8	1	3	1	8	12	14	3	17	14	24	6	25
E	8	7	1	3	5	1	1	1	4	4	3	12	13	5	12	18	4	19	2	25
F	2	1	3	6	1	2	4	7	8	2	1	5	5	10	14	6	13	14	30	20
G	2	4	9	6	5	9	3	4	1	9	4	6	4	4		19	13	23	15	30
H	1	7	5	4	8	1	4	3	3	6	5	1	7	3	17		14	12	21	16
I	4	9	8	3	2	4	9	7	5	1	1	6	8		9	5	14	15	23	19
J	6	4	1	8	3	5	1	4	4	8	5	5	16	4	16	5	6	8	16	13

Supplementary material S2

Table S1 the raw data of the optimization of single cell trapped (%) using different cell loading concentration (cells mL⁻¹).

Cell loading concentration (cells mL ⁻¹)	Single cell trapped (%)				
	#1	#2	#3	AVG	STV
10,000	12.5	13	12	12.5 ^a	0.5
12,500	25.5	25.5	25	25.3 ^b	0.3
15,000	31	28	34	31 ^c	3
17,500	15.5	14	14	14.5 ^a	0.9

Table S2 the raw data of the optimization of single cell trapped (%) using different settle time (min).

Settle time (min)	Single cell trapped (%)				
	#1	#2	#3	AVG	STV
1	11	7.5	12	10.2 ^a	2.4
3	16	14.5	17.5	16 ^b	1.5
5	32.5	32	32	32.2 ^c	0.3
7	19.5	17	16	17.5 ^a	1.8
9	4.5	4.5	5	4.7 ^d	0.3

Table S3 the raw data of efficiency of single cell isolation using limiting dilution and the microwell-based microfluidic device.

Techniques	Single cell trapped (%)										AVG	STV
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10		
Limiting dilution	4.5	6	5	6	5	5.5	6	3.5	5	5	5.2	0.8
The MBM device	21	28.5	23	25	28.5	17.5	15	29.5	27.5	27.5	24.3***	4.8

Table S4 the raw data of time spent processing using limiting dilution.

Tasks	Time (min)										AVG	STV
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10		
Cell preparation	10.2	5.2	4.5	10.3	5.7	6.1	5.3	4.5	5.8	5.2	6.3	2.2
Cell loading	3.2	3.4	5.1	4.2	3.3	3.3	2.5	4.2	2.4	3.2	3.5	0.8
Single cell assessment	31.2	15.2	12.3	12.5	12.8	22.3	18.1	31.2	12.3	13.5	18.14	7.6
Total time-spent processing	44.6	23.8	21.9	27	21.8	31.7	25.9	39.9	20.5	21.9	27.9	8.3

Table S5 the raw data of time spent processing using microwell-based microfluidic device.

Tasks	Time (min)										AVG	STV
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10		
Cell preparation	2.4	2.4	2.3	2.4	2.4	2.8	2.37	2.3	2.4	2.3	2.4	0.1
Cell loading	8	8.1	8.2	8.1	8.55	7.3	7.3	7	7	8.1	7.7	0.6
Single cell assessment	14.5	8.2	5.1	8.5	5	12	8.5	5.2	9.2	8.4	8.5	3
Total time-spent processing	24.9	18.7	15.6	19	15.95	22.1	18.17	14.5	18.6	18.8	18.6	3.1

Table S6 the raw data of single cell trapped correctness (%) between experienced and novice testers performing limiting dilution.

Testers	Single cell determination accuracy (%)						
	#1	#2	#3	#4	#5	AVG	STV
Experienced	100	83	90	83	70	85.3	9.8
Novice	85.7	91.8	100	100	100	73.2	16.3

Table S7 the raw data of single cell trapped correctness (%) between experienced and novice testers performing microwell-based microfluidic device.

Testers	Single cell determination accuracy (%)						
	#1	#2	#3	#4	#5	AVG	STV
Experienced	85.7	91.8	100	100	100	95.5	5.8
Novice	100	97.6	97.6	54.6	100	89.9	17.7