Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

An integrated high-throughput microfluidic circulatory fluorescence-activated cell sorting system (μ-CFACS) for the enrichment of rare cells

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1 The applied pressures for all the sorting procedures of the μ-CFACS system.

As described in the main manuscript, the pressure inlets and on-chip valves are selectively activated to choose the proper flow channels for each sorting process. As shown in Table. S1, the applied pressures are precisely controlled in all the sorting procedures of the μ-CFACS system. These values could be optimized for improving the enrichment performance of a specific target clinical cell type.

Table. S1 The values of applied pressures in all the sorting procedures of the μ-CFACS system. The unit is mbar for all the values.

Inlet / Procedure	Inlet A	Inlet B	Inlet C	Inlet D	Inlet E	Inlet F	Cylinder Valves
Primary sorting	550	0	275	0	0	0	5000
Dilute	500	0	0	0	0	0	5000
Recirculation	0	0	0	0	0	500	5000
Re-Sorting	550	0	325	0	0	0	5000

2 An example of calculating the hydraulic resistance and flow rate for the primary sorting procedure of the μ-CFACS system

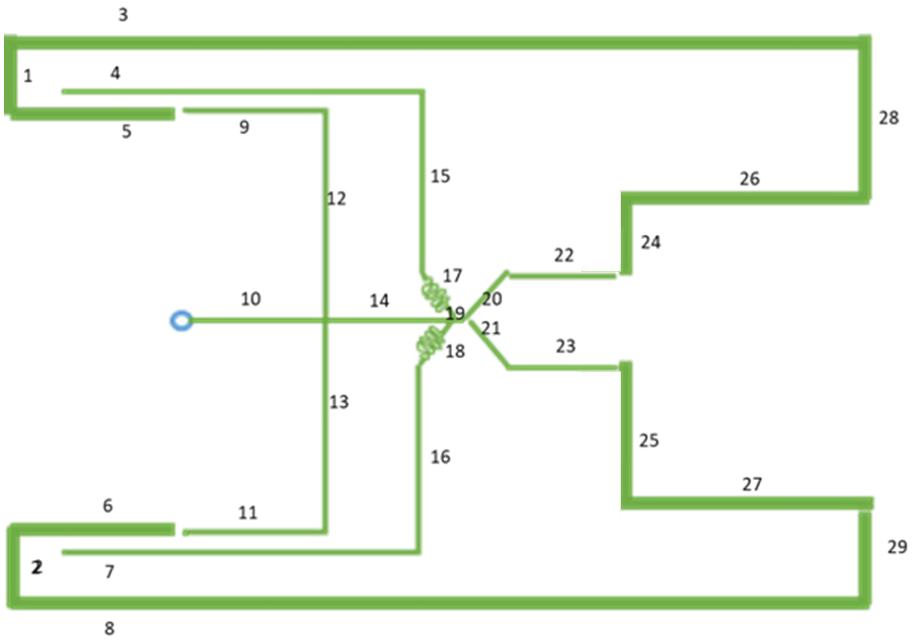

Fig. S1. Numbering of all the branch microfluidic channels in the PDMS sheet.

As the cross-section of microfluidic channels is fabricated in a rectangular shape in the developed cartridge. The theoretical hydraulic resistance of all the branch microfluidic channels as shown in Fig. S1 could be calculated by the following equation.[1]

$$R_{hyd\_theory} = \frac{12\eta L}{1 - 0.63(h/w)} \frac{1}{h^3 w} \quad (1)$$

As the theoretical formula does not consider the influence of hydrodynamic properties of solutions, materials of the cartridge, and interface effects, a correction parameter (α) of 0.69 was identified by experiments for transferring aqueous solutions in the microfluidic cartridge.

$$R_{hyd} = R_{hyd\_theory} \cdot \alpha \quad (2)$$

Given the specific dimensions of a microfluidic channel, its hydraulic resistance could be derived using Equation (1) and Equation (2). As an example, the dimensions and calculated hydraulic resistance for the active microfluidic channels in the primary sorting procedure are listed in Table S2.

Table S2. The dimensions and corresponding calculated hydraulic resistance for the active microfluidic channels in the primary sorting procedure.

No.	Length (mm)	Width (um)	Depth (um)	R_{hyd_theory} (Pa · s/m³)	R_{hyd_theory} (mbar · s/ul)	R_{hyd} (mbar · s/ul)
4	21.5	100	100	6.97E+12	70	48
7	21.5	100	100	6.97E+12	70	48
10	10	100	100	3.24E+12	32	22
14	8.5	100	100	2.76E+12	28	19
15	12	100	100	3.89E+12	39	27
16	12	100	100	3.89E+12	39	27
17	5	100	50	7.01E+12	70	48
18	5	100	50	7.01E+12	70	48
19	0.5	100	100	1.62E+11	2	1
21	3.5	100	100	1.14E+12	11	8
23	7	100	100	2.27E+12	23	16
25	9	200	100	7.88E+11	8	5
27	15	200	100	1.31E+12	13	9
29	6	200	100	5.26E+11	5	4

Hydraulic resistance distribution of microfluidic branches for the primary sorting procedure is provided in Fig. S2. The pressure of the sample focusing area is defined as Pre_{mid}, while the hydraulic resistances of the surrounding microfluidic channels are simplified into four combined hydraulic resistance. R_{samp} is the total hydraulic resistance from the sample inlet

until the focus point. R_{sh1} and R_{sh2} are respectively the hydraulic resistances from the two sheath inlets to the focus point. R_{samp} stands for the overall hydraulic resistance of the downstream microfluidic channels until the sample collection tank. The values of these four combined hydraulic resistances could be calculated by adding up the hydraulic resistances of related active microfluidic channels, as shown in Fig. S2(a).

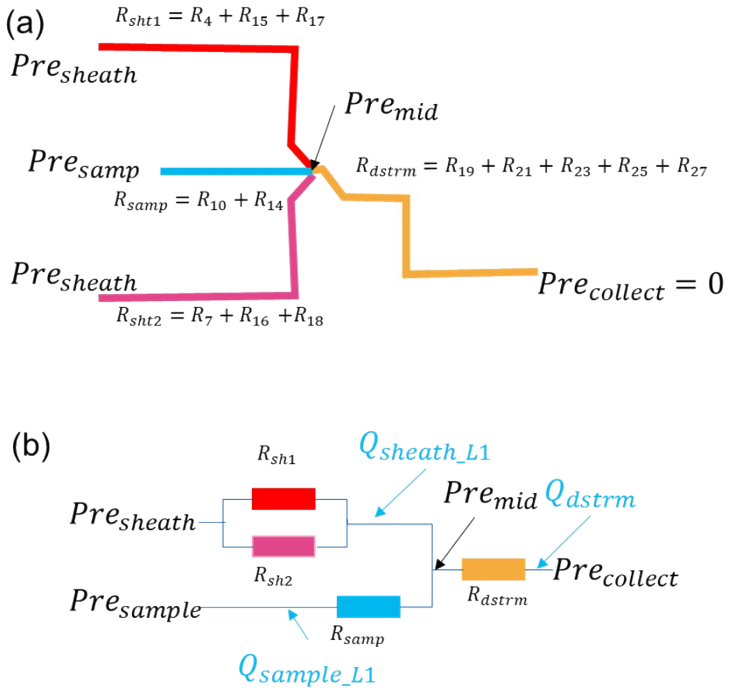

Fig. S2. (a) Simplified hydraulic resistance distribution of microfluidic branches for the primary sorting procedure. (b) The equivalent circuit diagram of the microfluidic network in (a).

The flow rates of microchannels follow Hagen-Poiseuille's law and Kirchhoff's lows as below,

$$Q_{dstrm} = Q_{sheath\_L1} + Q_{sample\_L1} \tag{3}$$

$$Q_{dstrm} = \frac{Pre_{mid} - Pre_{collect}}{R_{dstrm}} \tag{4}$$

$$Q_{sheath\_L1} = \frac{Pre_{sh} - Pre_{mid}}{R_{sht}} = \frac{Pre_{sh} - Pre_{mid}}{(R_{sht1} \cdot R_{sht2})/(R_{sht1} + R_{sht2})} = \frac{Pre_{sh} - Pre_{mid}}{R_{sht1}/2} \tag{5}$$

$$Q_{sample\_L1} = \frac{Pre_{samp} - Pre_{middle}}{R_{samp}} \tag{6}$$

By combining Equation (3), Equation (4), Equation (5), and Equation (6), Pre_{mid} could be calculated from the following equation.

$$\frac{Pre_{mid} - Pre_{collect}}{R_{dstrm}} = \frac{Pre_{sh} - Pre_{mid}}{R_{sht1}/2} + \frac{Pre_{samp} - Pre_{middle}}{R_{samp}} \tag{7}$$

Q_{sample_L1} and Q_{sheath_L1} could be then be obtained by applying Pre_{mid} into Equation (5) and Equation (6). These data could further be used for performance calculation as described in

the main manuscript.

Flow rates and hydraulic resistances of other μ-CFACS procedures could be calculated similarly.

3 Discussion on time efficiency of the μ-CFACS system and a single primary sorting procedure after additional dilution.

Generally, to collect a sample with desired purity ($^{Pur_{desired}}$) after sorting, the non-target cells number per switching volume should be reduced by $^{Dr_{desired}}$ times from $^{n_{non\_target\_L1}}$ to $^{n_{non\_target\_desired}}$. To achieve this through a single primary sorting procedure, the sample volume should be diluted by $^{Dr_{desired}}$ times. Hence the time cost for the single primary sorting procedure would be written as,

$$t_{single\_sort} = Dr_{desired} \cdot t_{L1} \tag{8}$$

To achieve $^{Dr_{desired}}$ by adding ^{n} rounds of dilution and resorting procedures after the primary sorting procedure, $^{Dr_{desired}}$ could also be written into

$$Dr_{desired} = \prod_{1}^{n} x_n \tag{9}$$

where ^{x_n} is the dilution ratio for the ^{n}th round of the re-sorting procedure, the value of which is larger than 1. For an ideal μ-CFACS process, where there is no target loss for all the ^{n} rounds of re-sorting procedures, the time cost for the ^{n}th round of re-sorting procedure could be given by

$$t_{sort\_n} = Cr \cdot t_{L1} \cdot x_n \tag{10}$$

Where ^{Cr} is the cut-out ratio of collected sample volume when compared with total sample volume during the primary sorting procedure, the value of which could be given by

$$Cr = \frac{n_{sort\_L1} \cdot V_{switch\_sample}}{V_{L1}} \tag{11}$$

Assuming the dilution and recirculation could be completed in an extremely short time, which is close to 0 seconds. The minimal total time of the whole μ-CFACS process ($^{t_{\mu-CFACS}}$) could be calculated by

$$t_{\mu-CFACS} = t_{L1} + \sum_{1}^{n} t_{sort\_n} \tag{12}$$

From Equation (10) and Equation (12), $^{t_{\mu-CFACS}}$ would be rewritten into

$$t_{\mu-CFACS} = t_{L1} + Cr \cdot t_{L1} \sum_{1}^{n} x_n \tag{13}$$

Since the geometric mean of a non-empty data set of positive numbers is always at most

their arithmetic mean, which is stated that

$$\frac{1}{n} \cdot \sum_{1}^{n} x_n \geq \prod_{1}^{n} x_n^{\frac{1}{n}} \tag{14}$$

By Equation (13) and Equation (14), the value of $t_{\mu-CFACS}$ should follow that

$$t_{\mu-CFACS} \geq t_{L1} + Cr \cdot t_{L1} \cdot n \cdot \prod_{1}^{n} x_n^{\frac{1}{n}} \tag{15}$$

and that equality holds if and only if $x_1 = x_2 = \cdots = x_n = Dr_{desired}^{\frac{1}{n}}$, which means that the collected sample is uniformed diluted at a constant ratio for the additional n rounds of dilution and resorting procedures after the primary sorting procedure. In this case, $t_{\mu-CFACS}$ would be rewritten into

$$t_{\mu-CFACS} = t_{L1} + Cr \cdot t_{L1} \cdot n \cdot Dr_{desired}^{\frac{1}{n}} \tag{16}$$

The minimal value of $t_{\mu-CFACS}$ would be obtained as bellow when n is equal to $ln(Dr_{desired})$.

$$t_{\mu-CFACS\_min} = (1 + Cr \cdot ln(Dr_{desired}) \cdot e) \cdot t_{L1} \tag{17}$$

Where e is the exponential constant. Comparing Equation (8) and Equation (17), when the single primary sorting procedure (t_{single_sort}) is less than the minimum value of the whole μ-CFACS process, rather than adopting a μ-CFACS process, a single primary sorting procedure should be preferred. The situation could also be stated as

$$Cr > \frac{Dr_{desired} - 1}{e \cdot ln(Dr_{desired})} \tag{18}$$

4 Typical FCM scatters of the original cell mixture and samples collected after three continuous sorting procedures.

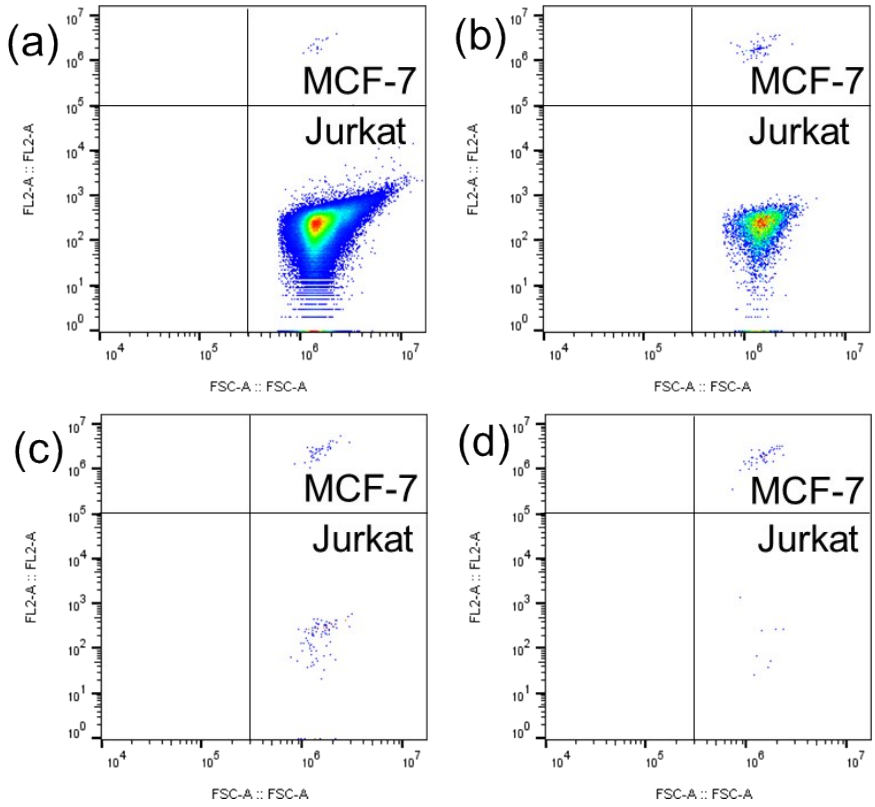

Fig. S3. Typical FCM scatters for cell mixtures of fluorescently labeled MCF-7 and Jurkat cells: (a) the original cell mixture, (b) the cell mixture collected after the 1st sorting, (c) the cell mixture collected after the 2nd sorting,(d) the cell mixture collected after the 3rd sorting.

Typical FCM scatters of the original cell mixture and samples collected after three continuous sorting procedures are independently given in Fig. S3(a)-(d). Under our experimental conditions, the typical purity of the target MCF-7 cells was individually observed to be ~ 0.009 %, ~ 0.9 %, ~ 30 %, and ~ 80 % for the original sample and samples collected after the 1st, 2nd, and 3rd sequential sorting procedure.