Electronic Supplementary Material (ESI) for Lab on a Chip
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# SUPPLEMENTARY MATERIAL

## Integration of a microfluidic chip with a size-based cell bandpass filter for reliable isolation of single cells

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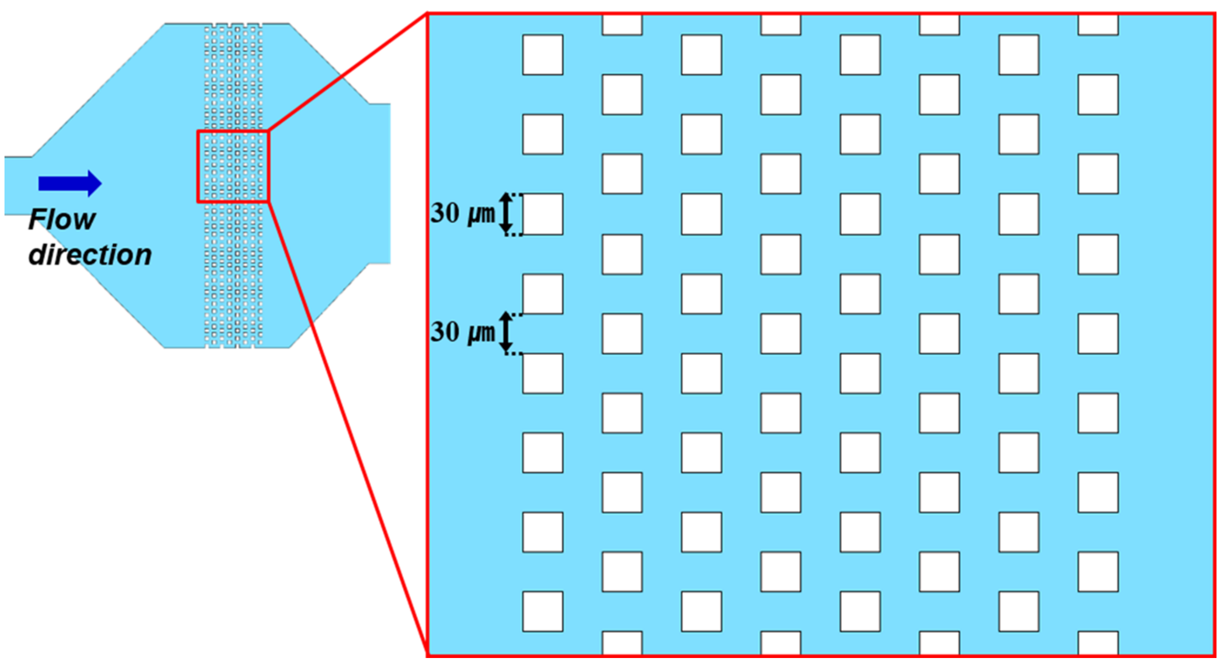
1. The design and dimensions of the microfluidic array chip for single cell isolation.

(a) Design and dimensions of the on-chip pre-filter with a cut-off size of 30 µm.



(b) The design and dimensions of the cell-trapping site.

As mentioned in the main text, the cell-trapping site is composed of the cell trap region with a flow fraction-based hydrodynamic filtering network (i.e., flow fraction-based cell filter). To provide the details of the design process and as a guideline for researchers who are interested in single-cell isolation, each step in the process is given below.

i) Step 1: Determination of the cut-off size and the channel height

The cut-off size (i.e., the space between the pillars of the pre-filter) and the channel height were designed to be 30 μm each, based on the size distribution of these cells, to prevent channel clogging by influent cells (see Fig. S1 below).

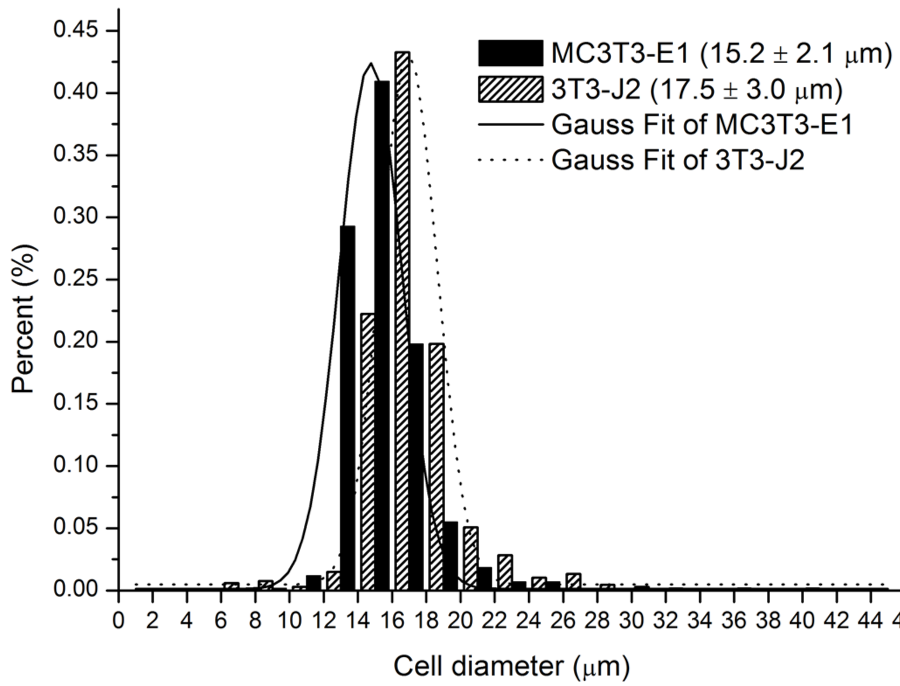



**Fig. S1** Size distribution profiles of 3T3-J2 and MC3T3-E1 cell lines

ii) Step 2: Determination of a band range (i.e., the cut-off size and the cut-on size)

The cut-on size [i.e., theoretically twice the width ($w_b$) of the stream entering the bypass channels in the flow fraction network (see, Fig. 1c of the revised manuscript)] was designed to be 0, 16, or 18 μm, in order to investigate the effects of influent cell size on single-cell trapping performance (i.e., trapping efficiency). Therefore, the band range was designed to be 0–30 μm, 16–30 μm, and 18–30 μm, respectively. Note that a microfluidic chip with a band range of 0–30 μm consists of an on-chip pre-filter and a cell trap region without a flow fraction-based filter.

iii) Step 3: Design of a cell trap

$W_T$, $W_{T1}$, $L_{T1}$, and $L_T$ of a cell trap (see Fig. A below) were designed to accommodate a single cell and to create large differences in the hydraulic resistances of a cell trap upon capturing of a cell. Dimensions (i.e., $W_T$, $W_{T1}$, $L_{T1}$, and $L_T$) of the cell trap should thus be similar to the cell size. In the cell trap, the length of the bypass channel (i.e., the sum of $L_{TB2}$, $L_T$, and $L_{TB3}$), which regulates the width ($w_{t,b}$) of the stream in an empty trap, was designed for a $w_{t,b}$ of 21 μm, allowing cells to efficiently enter the cell trap [i.e., $r_{cell,min} > W_M - w_{t,b}$ (as shown in Fig. 1c of the manuscript), where $r_{cell,min}$ is 9 μm for a band range of 18–30 μm and the width ($W_M$) of the main channel is 30 μm]. Using a hydraulic-electric circuit analogy, the bypass channel length was designed to be 413 μm [see our previous work to determine flow division (i.e., the ratio of the bypassing flow rate to the main flow rate) at the branch point].[1] Next, 20 cell traps were arranged sequentially, based on the field of view of a charge-coupled device (CCD). Short lengths of $L_{TB1}$, $L_{TB4}$, and $L_{G1}$ were designed to minimize the hydraulic resistance.

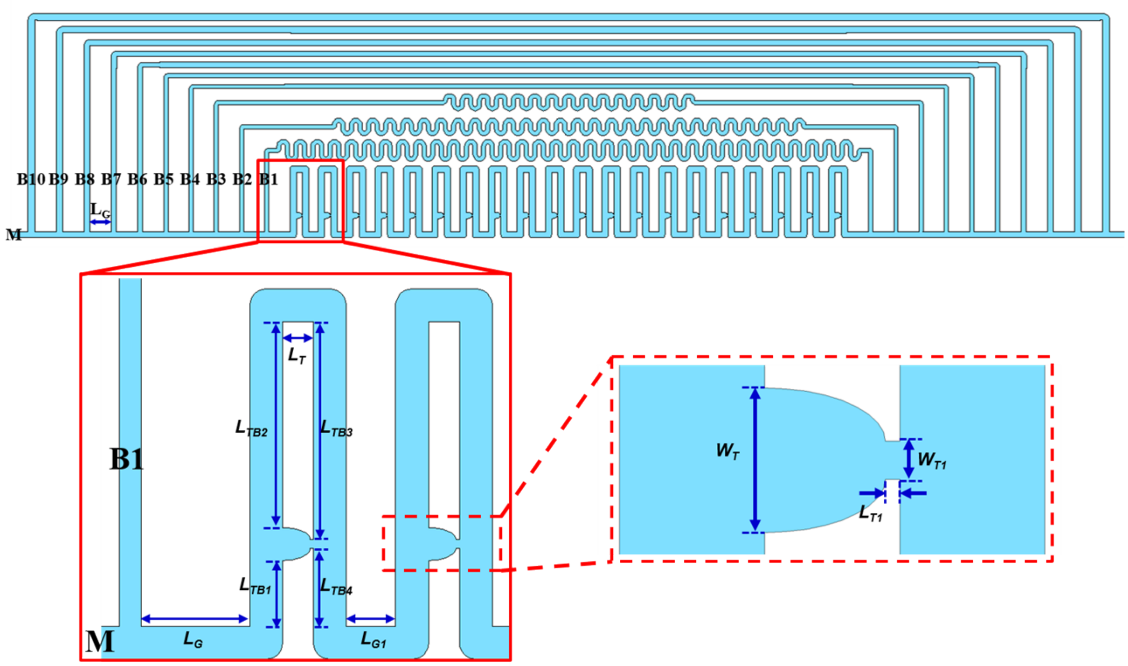



**Fig. A** Enlarged views of the cell-trapping site

iv) Step 4: Determination of the dimensions of bypass channels in the flow fraction network

To determine the widths and the lengths of bypass channels corresponding to a $w_b$ of 8 or 9 μm (i.e., band ranges of 16–30 and 18–30 μm, respectively), the electric circuit analogy shown below was applied.[2] The proposed cell-trapping site can be replaced by an equivalent electric circuit (see Fig. B below).

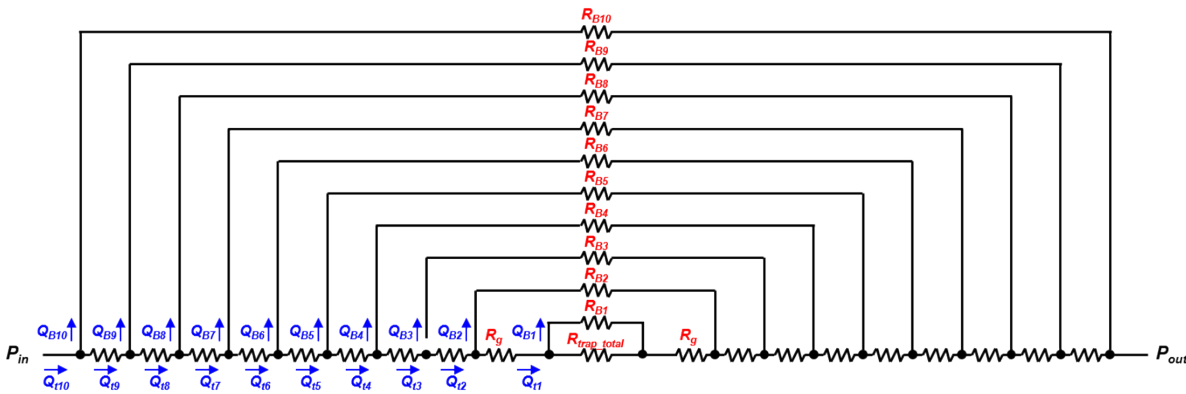



**Fig. B** Equivalent electric circuit of the cell-trapping site

Assuming that the velocity profile is a two-dimensional parabola [note that the velocity profile can be assumed to be a two-dimensional parabola if the aspect ratio of the channel height ($H$) to width ($W_M$) is larger than ~1],[3] the $Q_{tn}/Q_{Bn}$ ratio ($n$ = 1, 2, …, 10) can be replaced as follows:[1]

$$\frac{Q_{tn}}{Q_{Bn}} = \left( \frac{1}{3\left(\frac{w_b}{w_M}\right)^2 - 2\left(\frac{w_b}{w_M}\right)^3} \right) \quad \text{(eqn. S1)}$$

Therefore, to obtain the desirable $w_b$ of 8 and 9 μm, the $Q_{tn}/Q_{Bn}$ ratio should be ~5.70 and ~4.63, respectively. The value of $R_{trap\_total}$ can be calculated using equation S2 to determine the hydraulic resistance of cell traps with a rectangular cross-sectional shape, because the dimensions (i.e., width and the length) of the cell trap region are given above in Step 3.

$$R_B = \frac{f_{Re} \times \mu \times L_B}{2D_h^2 \times A_B} \quad \text{(eqn. S2)}$$

where $f_{Re}$ is the laminar friction constant, $L_B$ is the bypass channel length, $D_h$ is the hydraulic diameter, and $A_B$ is the channel cross-sectional area. The mathematical definitions of $f_{Re}$, $D_h$, and $A_B$ were given in our previous work.[1] Next, the hydraulic resistance $R_{Bn}$ ($n$ = 2, 3, …, 10) can be expressed by the following recurrence formula (equation S3) through hydraulic analogy to Kirchhoff's voltage law (KVL):

$$R_{Bn} = \left(\frac{Q_{tn}}{Q_{Bn}} - 1\right)(R_n + 2R_g), \qquad R_{B1} = \left(\frac{Q_{tn}}{Q_{Bn}} - 1\right)R_{trap\_total} \tag{eqn. S3}$$

where $R_{Bn}$ is the hydraulic resistance of a bypass channel, $R_g$ is the hydraulic resistance of a gap channel, and $R_1 = R_{B1} \| R_{trap\_total}$, $R_2 = R_{B2} \| (R_1 + 2R_g)$, $R_3 = R_{B3} \| (R_2 + 2R_g)$, …, $R_{10} = R_{B10} \| (R_9 + 2R_g)$ [note that symbol "‖" indicates a shorthand notation for a parallel combination of electric resistors]. The equivalent hydraulic resistance $R_n$ ($n$ = 2, 3, …, 10) can then be expressed as follows:

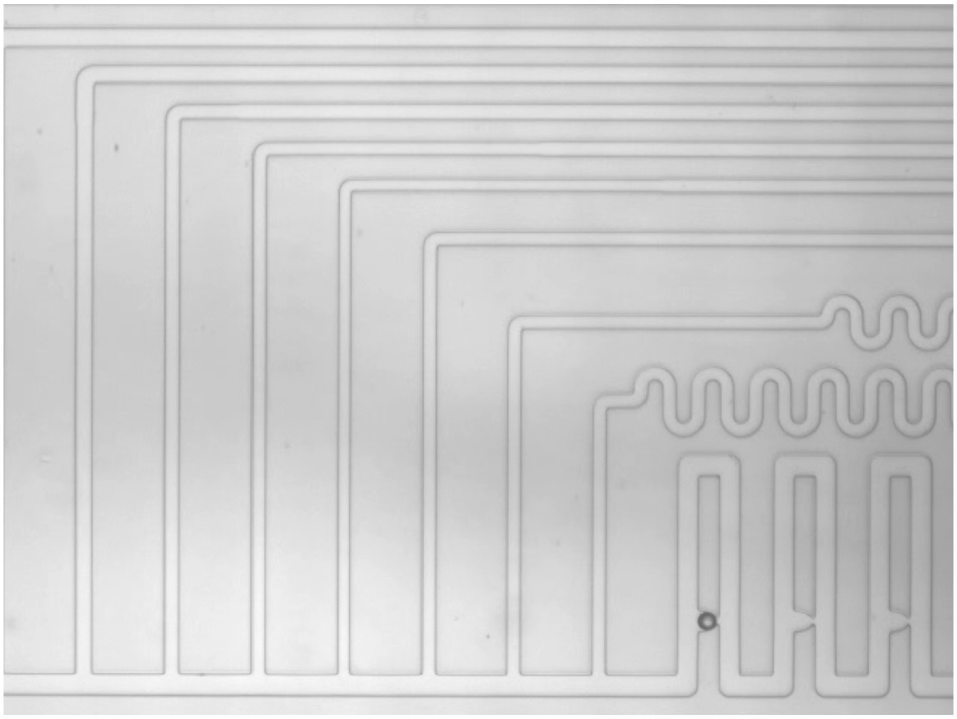
$$R_n = \frac{R_{Bn}(R_{n-1} + 2R_g)}{R_{n-1} + 2R_g + R_{Bn}}, \qquad R_1 = \frac{R_{B1} R_{trap\_total}}{R_{B1} + R_{trap\_total}} \tag{eqn. S4}$$

By solving equations S3 and S4 simultaneously, the value of $R_{Bn}$ can be calculated. Finally, the widths and lengths of bypass channels can be determined using equation S2. Below, the dimensions of cell-trapping sites required to achieve a band range of 18–30 μm are provided.

| | **Width [μm]** | **Length [μm]** |
|---|---|---|
| **M (main channel)** | 30 | - |
| **B1 (bypass channel)** | 20 | 9944 |
| **B2** | 20 | 8075 |
| **B3** | 20 | 6610 |
| **B4** | 18 | 2200 |
| | 20 | 2663 |
| **B5** | 20 | 2200 |
| | 22 | 2999 |
| **B6** | 22 | 1700 |
| | 24 | 3839 |
| **B7** | 24 | 1530 |
| | 26 | 4243 |
| **B8** | 28 | 6231 |
| **B9** | 30 | 4000 |
| | 32 | 2607 |
| **B10** | 32 | 2000 |
| | 34 | 4987 |

| | **Length [μm]** |
|---|---|
| $\mathbf{L_{TB1}}$ | 60 |
| $\mathbf{L_{TB2}}$ | 187 |
| $\mathbf{L_{TB3}}$ | 198 |
| $\mathbf{L_{TB4}}$ | 71 |
| $\mathbf{L_{T}}$ | 28 |
| $\mathbf{L_{G}}$ | 100 |
| $\mathbf{L_{G1}}$ | 100 |
| $\mathbf{W_{T}}$ | 30 |
| $\mathbf{W_{T1}}$ | 8 |
| $\mathbf{L_{T1}}$ | 3 |
| | |

2. A video (Movie1) showing the filtering of smaller-sized beads (measuring 15 μm in diameter) and the sequential trapping of larger-sized beads (measuring 25 μm in diameter).



# References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