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 \( \mu \text{m} \) 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](image)

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 \( \mu \text{m} \), 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 \( \mu \text{m} \), 16–30 \( \mu \text{m} \), and 18–30 \( \mu \text{m} \), respectively. Note that a microfluidic chip with a band range of 0–30 \( \mu \text{m} \) 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 $\mu$m, 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 $\mu$m for a band range of 18–30 $\mu$m and the width ($W_M$) of the main channel is 30 $\mu$m]. Using a hydraulic-electric circuit analogy, the bypass channel length was designed to be 413 $\mu$m [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](image-url)
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 \( \mu \text{m} \) (i.e., band ranges of 16–30 and 18–30 \( \mu \text{m} \), respectively), the electric circuit analogy shown below was applied. The proposed cell-trapping site can be replaced by an equivalent electric circuit (see Fig. B below).

![Equivalent electric circuit of the cell-trapping site](image)

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 \( \sim 1 \)],\(^3\) the \( Q_{tn}/Q_{Bn} \) ratio \((n = 1, 2, \ldots, 10)\) can be replaced as follows:\(^1\)

\[
\frac{Q_{tn}}{Q_{Bn}} = \left( \frac{1}{3 \left( \frac{w_b}{w_M} \right)^2 - 2 \left( \frac{w_b}{w_M} \right)^3} \right)
\]  

(eqn. S1)

Therefore, to obtain the desirable \( w_b \) of 8 and 9 \( \mu \text{m} \), the \( Q_{tn}/Q_{Bn} \) ratio should be \( \sim 5.70 \) and \( \sim 4.63 \), respectively. The value of \( R_{\text{trap, total}} \) 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.

\[
R_B = \frac{f_{Re} \times \mu \times L_B}{2D_b^2 \times A_B}
\]  

(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 \( R_{Bn} (n = 2, 3, \ldots, 10) \) can be expressed by the following recurrence formula (equation S3) through hydraulic analogy to Kirchhoff’s voltage law (KVL):

\[
R_{Bn} = \left( \frac{Q_{tn}}{Q_{Bn}} - 1 \right) \left( R_n + 2R_g \right), \quad R_{B1} = \left( \frac{Q_{tn}}{Q_{Bn}} - 1 \right) R_{trap\_total}
\]

(eqnn. S3)

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

\[
R_n = \frac{R_{Bn}(R_{n-1} + 2R_g)}{R_{n-1} + 2R_g + R_{Bn}}, \quad R_1 = \frac{R_{B1}R_{trap\_total}}{R_{B1} + R_{trap\_total}}
\]

(eqnn. 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 \( \mu \)m are provided.

<table>
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<tr>
<th></th>
<th>Width [( \mu )m]</th>
<th>Length [( \mu )m]</th>
<th>Length [( \mu )m]</th>
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<tr>
<td><strong>M</strong> (main channel)</td>
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<td>-</td>
<td><strong>L_{TB1}</strong></td>
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<tr>
<td><strong>B1</strong> (bypass channel)</td>
<td>20</td>
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<td>6610</td>
<td><strong>L_{TB4}</strong></td>
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<td>18</td>
<td>2200</td>
<td><strong>L_T</strong></td>
</tr>
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<td>2663</td>
<td><strong>L_G</strong></td>
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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