Supplementary Information

A Microfluidic Chip with Serpentine Channel Enabling High-throughput Cell Separation Using Surface Acoustic Waves

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Device setup

The sample flow and sheath flow driven by syringe pumps were injected into PDMS microchannel, and the samples after separation were collected through sterile tubing in centrifuge tubes (blue path). The required signal was generated by a signal generator and amplified by a RF power amplifier, then the signal was divided into two channels and applied to two interdigital transducers, respectively (black path). Observation and image collection were performed on the microscopic imaging platform. A lamp source excited fluorescent tags on polystyrene microspheres and *E. coli* (blood cells were observed in bright field) through the microscope objective, and images (sequences) were captured via camera (orange path). The collected images were further processed and analyzed on PC (purple path).

Figure S1. Diagram of device setup and separation workflow (a) Instrumentation schematic for cell separation, and data collection. (b) Side view of the experimental setup.
Table S1 Summary for separation techniques using microfluidic tools.

<table>
<thead>
<tr>
<th>Separation technique</th>
<th>Separation principle</th>
<th>Sample composition</th>
<th>Throughput/flow rate</th>
<th>Target cell recovery</th>
<th>Target cell Purity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Passive separation techniques:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pillar array structure</td>
<td>Size, deformability</td>
<td>Diluted whole blood</td>
<td>1000 μm/s</td>
<td>~99%</td>
<td>~99%</td>
<td>[1]</td>
</tr>
<tr>
<td>Hydrodynamic filtration</td>
<td>Size</td>
<td>Leukocytes and RBCs</td>
<td>20 μL/min</td>
<td>—</td>
<td>increased ~29-fold</td>
<td>[2]</td>
</tr>
<tr>
<td>Inertia focusing</td>
<td>Size, shape, deformability</td>
<td><em>E. coli</em> mixed with diluted whole blood</td>
<td>15 μL/min</td>
<td>62%</td>
<td>99.87%</td>
<td>[3]</td>
</tr>
<tr>
<td><strong>Active separation techniques:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSAW</td>
<td>Size, density, compressibility</td>
<td><em>E. coli</em> and PBMCs</td>
<td>0.5 μL/min</td>
<td>—</td>
<td>95.56%</td>
<td>[4]</td>
</tr>
<tr>
<td>taSSAW</td>
<td>Size, density, compressibility</td>
<td><em>E. coli</em> and RBCs</td>
<td>1 μL/min</td>
<td>&gt;98%</td>
<td>&gt;97%</td>
<td>[5]</td>
</tr>
<tr>
<td>TSAW</td>
<td>Size, density, compressibility</td>
<td>3 &amp; 10 μm particles</td>
<td>25 μL/h</td>
<td>Close to 100%</td>
<td>Close to 100%</td>
<td>[6]</td>
</tr>
<tr>
<td>SSAW+TSAW</td>
<td>Size, density, compressibility</td>
<td>RBCs &amp; U87 glioma cells</td>
<td>0.3μL/min</td>
<td>—</td>
<td>90%</td>
<td>[7]</td>
</tr>
<tr>
<td>DEP</td>
<td>Size, polarizability</td>
<td><em>E. coli</em> mixed with diluted whole blood</td>
<td>0.5 μL/min</td>
<td>87.2%</td>
<td>Close to 100%</td>
<td>[8]</td>
</tr>
<tr>
<td>Magnetophoresis</td>
<td>Size, magnetic susceptibility</td>
<td><em>E. coli</em> and RBCs</td>
<td>25 μL/h</td>
<td>78%</td>
<td>&gt;99%</td>
<td>[9]</td>
</tr>
<tr>
<td>Optical</td>
<td>Size, refractive index, polarizability</td>
<td>Polymer and silica spheres</td>
<td>30 μm/s</td>
<td>—</td>
<td>—</td>
<td>[10]</td>
</tr>
<tr>
<td>Serpentine-SSAW</td>
<td>Size, density, compressibility</td>
<td><em>E. coli</em> and RBCs</td>
<td>2.6 μL/min</td>
<td>97.50%*</td>
<td>98.02%*</td>
<td>This work</td>
</tr>
</tbody>
</table>

*In this work, the cell recovery and purity were calculated without considering cells did not belong to either *E.coli* domain or RBCs domain.*
Fluorescent image details

In this paper, we used fluorescent polystyrene particles and GFP-\textit{E. coli} to demonstrate separation performance of the acoustofluidic device, and the distribution of particles/cells in serpentine microchannel was semi-quantitatively analyzed by microscopic fluorescent image sequences. Meanwhile, we stacked and averaged the consecutive image sequences, and the

Figure S2. Fluorescent image sequence for 5 μm red fluorescent polystyrene particles in serpentine microchannel. The exposure time was 2.3 s.
results were shown in Figure 3(a)(b) and Figure 4(b). Each frame of fluorescent images was illustrated as follow.

Figure S3. Fluorescent image sequence for 1 μm green fluorescent polystyrene particles in serpentine microchannel. The exposure time was 3.8 s.
Figure S4. Fluorescent image sequence for 5 μm red fluorescent polystyrene particles in single-channel device. The exposure time was 2.7 s.
Figure S5. Fluorescent image sequence for GFP-expressing *E. coli* in serpentine microchannel. The exposure time was 5 s.
The serpentine microfluidic design with three subsections needs two 180° turns. In our design, these two turns were located outside the acoustic separation area to avoid that particles or cells deviated from previous trajectories when they flowed through the turns. Besides, no obvious mixing between sample flow and sheath flow was observed at the turns whether SSAW field exited or not.

Figure S6. Distribution of particles (5 μm with red fluorescence and 1 μm with green fluorescence) at the corners of serpentine microchannel while SAW was on.
RBCs and E. coli domain in flow cytometry scatter plot

Before further analysis for cell population components in samples, the domains for RBCs and E. coli in 2D flow cytometry scatter plot was determined by the testing results of pure cell samples. As shown in Figure S3, E. coli and RBCs could be clearly distinguished by their difference on side scatter (SSC-A) and forward scatter (FSC-A). The pattern of RBCs was relatively concentrated, with more than 99% RBCs were included by an elliptical domain; and the most of E. coli (95.2%) were contained in a drop-shaped domain while the pattern of E. coli was more scattered.

![Flow cytometry results of pure RBCs and E. coli.](image)
Simulation details

The numerical study of acoustic pressure field and particle trajectories were implemented on the finite element software *COMSOL Multiphysics* 5.5. We used the following 2 steps to analyze separation performance of the acoustofluidic device in COMSOL:

1. **Analysis of acoustic pressure field**

The acoustofluidic device (including LiNbO$_3$ substrate, gold interdigital transducers, PDMS and fluids) were modeled in the section plane of microchannel ($x$-$z$ plane) according to actual geometric parameters of devices (Figure S8).

a). PDMS domain

The lid of PDMS channel are several millimeters in height. Related works demonstrated that almost all transmitted wave energy was absorbed in the PDMS, i.e. no waves can reflect from top boundary back to PDMS-liquid interface with the thickness of PDMS lid[11][12]. Therefore, we introduced a perfect matching layer to describe the low-reflection boundary condition. In this step, we used “Solid Mechanics” interface to describe PDMS and considered PDMS as a linear elastic material[13]. The governing equations of PDMS domain were:

\[ -\rho_{PDMS} (1 - i\mu_{PDMS}) \omega^2 u_s = \nabla \cdot \sigma_s \text{ \ (* MERGEFORMAT 1)} \]

\[ \sigma_s = \rho_{PDMS} \left[c_S^2 \left( \nabla u_s + (\nabla u_s)^T \right) + \left(c_L^2 - 2c_S^2 \right)(\nabla \cdot u_s)I \right] \text{\ (* MERGEFORMAT 2)} \]

where $\sigma_s$ and $u_s$ were stress tensors and displacement field of PDMS domain, $\rho_{PDMS}$ and $\mu_{PDMS}$ were the density and damping coefficient of PDMS[14]. $c_L$ and $c_T$ were the velocities of the longitudinal and shear waves in PDMS, respectively. $I$ was the unit tensor.

b). Water domain

Inside the PDMS microchannel, water domain was modeled by “Thermoviscous Acoustics, Frequency Domain” interface. the governing equations consisted of three parts,

\[ i\omega \rho_f = -\rho_f \nabla \cdot v_f \text{ \ (* MERGEFORMAT 3)} \]
the continuity equation, where \( \rho_f \) and \( \rho_f^1 \) were the density of water and its 1st-order harmonic perturbation, and \( \mathbf{v}_f \) was 1st-order velocity field;

\[
\begin{align*}
  i\omega \rho_f \mathbf{v}_f &= \nabla \cdot \mathbf{\sigma}_f \\
  \mathbf{\sigma}_f &= -p \mathbf{I} + \mu_f \left( \nabla \mathbf{v}_f + \nabla \mathbf{v}_f^T \right) + \left( \eta_f + \frac{1}{3} \mu_f \right) \left( \nabla \cdot \mathbf{v}_f \right) \mathbf{I}
\end{align*}
\]

(4)

the momentum equation, where \( \mathbf{\sigma}_s \) was stress tensors of water domain \( \mu_f \) and \( \eta_f \) were the shear and bulk viscosities of water, and \( p \) was acoustic pressure;

\[
\begin{align*}
  i\omega \left( \rho_f C_p T - T_0 \alpha_f \mathbf{p}_f \right) &= -\nabla \cdot \left( -k_f \nabla T \right)
\end{align*}
\]

(5)

the energy conservation equation, where \( C_p, \alpha_f \) and \( k_f \) were specific heat capacity, thermal expansion coefficient and thermal conductivity, respectively, and \( T \) was temperature (\( T_0 \) took the ambient value 293.15 K).

c). LiNbO\(_3\) substrate and IDTs

The LiNbO\(_3\) substrate and IDTs were built by “Acoustic-Piezoelectric Interaction, Frequency Domain” interface, which included “Solid Mechanics” and “Electrostatics” models. Considering piezoelectric theories were mature\(^{[14]}\) and described in many works, the governing equations (followed COMSOL Physics Library) were not reiterated here. Besides, because the actual size of LiNbO\(_3\) substrate is much larger than in this model, perfect matching layers are added on both sides of LiNbO\(_3\) domain to ignore reflections.

d). Boundary conditions

In this model, the outer side boundaries of solid domain were both free, i.e., no-stress condition for \( \mathbf{\sigma}_s \) is applied along exterior boundaries. \( \mathbf{n} \) was the normal vector.

\[
\mathbf{n} \cdot \mathbf{\sigma}_s = 0
\]

(6)

On the interior boundaries, continuity of velocity is implemented as a boundary condition on \( \mathbf{v}_f \) in the fluid domain imposed by the velocity in solid domain. Considering the thermoviscous acoustic-structure boundary, the fluid-solid continuity described by

\[
\mathbf{v}_f = i\omega \mathbf{u}_s, -\mathbf{n} \cdot \left( -k_f \nabla T \right) = 0
\]

(7)

At the solid-solid interface between PDMS and LiNbO\(_3\), continuity of stress and displacement were implemented as boundary conditions.

\[
\mathbf{u}_L = \mathbf{u}_s, \quad \mathbf{\sigma}_L = \mathbf{\sigma}_s
\]

(8)

where \( \mathbf{\sigma}_L \) and \( \mathbf{u}_L \) were stress tensors and displacement of LiNbO\(_3\).

e). Mesh setup

In this simulation, each material domain was meshed by free triangular elements. To fully resolve the acoustic boundary between PDMS and water, we introduced a thin viscous boundary layer with width \( \delta \).\(^{[13]}\)
\[ \delta = \sqrt{\frac{2\eta_f}{\rho_f \cdot \omega}} = 0.15 \mu m, \ \omega = 2\pi \cdot 12.8 MHz \ \text{(* MERGEFORMAT (9))} \]

The maximum mesh sizes of PDMS and water domain except boundary areas were 6μm (much smaller than wavelengths of shear waves in these areas) and 3μm (20δ). Element sizes in the substrate was shorter than \( \lambda/15=20\mu m \).

2. Particle trajectories simulation

Modeled on horizontal plane (x-y plane), and applied the “Laminar Flow” and “Particle Tracing” interface. In the “Particle Tracing” Module, each particle was treated as an independent particle governed by Newton’s law of motion. By coupling the stokes drag field (Equation(5) in paper) with the acoustic pressure field (Equation(4) in paper) solved from analysis of acoustic pressure field, the trajectories of particles in SSAW field could be calculated. The numerical simulation result was shown in Video 1.

All relevant parameters used in the numerical study are listed in Table S2. The parameters were given at room temperature (T=293.15 K), and other unspecified parameters taken from COMSOL Material Library.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>$\rho_f$</td>
<td>998 kg/m$^3$</td>
</tr>
<tr>
<td>Shear viscosity</td>
<td>$\eta_f$</td>
<td>$8.93 \times 10^{-4}$ Pa·s</td>
</tr>
<tr>
<td>Bulk viscosity</td>
<td>$\mu_f$</td>
<td>$2.47 \times 10^{-3}$ Pa·s</td>
</tr>
<tr>
<td>Longitudinal wave velocity</td>
<td>$c_f$</td>
<td>1497 m/s</td>
</tr>
<tr>
<td>Specific heat capacity</td>
<td>$C_p$</td>
<td>4183 J/kg·K</td>
</tr>
<tr>
<td>Thermal expansion coefficient</td>
<td>$\alpha_f$</td>
<td>2.97$ \times 10^{-4}$/K</td>
</tr>
<tr>
<td>Thermal conductivity</td>
<td>$k_f$</td>
<td>0.603 W/m·K</td>
</tr>
<tr>
<td>Compressibility</td>
<td>$\beta_f$</td>
<td>$4.48 \times 10^{-10}$/Pa</td>
</tr>
<tr>
<td><strong>Particle (Polystyrene)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>$\rho_p$</td>
<td>1050 kg/m$^3$</td>
</tr>
<tr>
<td>Compressibility</td>
<td>$\beta_p$</td>
<td>$2.59 \times 10^{-10}$/Pa</td>
</tr>
<tr>
<td><strong>PDMS (10:1)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>$\rho_{PDMS}$</td>
<td>920 kg/m$^3$</td>
</tr>
<tr>
<td>Longitudinal wave velocity</td>
<td>$c_L$</td>
<td>1030 m/s</td>
</tr>
<tr>
<td>Shear wave speed</td>
<td>$c_S$</td>
<td>100 m/s</td>
</tr>
<tr>
<td>Damping coefficient$^{[13]}$</td>
<td>$\mu_{PDMS}$</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Substrate (128°Y-X LiNbO$_3$)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sound velocity</td>
<td>$c_{SAW}$</td>
<td>3953 m/s</td>
</tr>
<tr>
<td><strong>Else</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wavelength (set by IDTs)</td>
<td>$\lambda$</td>
<td>300 $\mu$m</td>
</tr>
<tr>
<td>Width of microchannel</td>
<td>$W$</td>
<td>300 $\mu$m</td>
</tr>
<tr>
<td>Height of microchannel</td>
<td>$H$</td>
<td>25 $\mu$m</td>
</tr>
<tr>
<td>Length of acoustic separation region</td>
<td>$L$</td>
<td>5 mm</td>
</tr>
<tr>
<td>Voltage amplitude</td>
<td>$V_{pp}$</td>
<td>36 V</td>
</tr>
</tbody>
</table>
Bacterial activity and function detection

To verify that this acoustofluidic separation technique can be applied to clinical testing, we tested the activity and function of bacterial cells. In subsequent testing, we set up a control group to compare with the sample after acoustofluidic separation. In the control group, sample was introduced in the same conditions, but without RF signal (SAW off).

Firstly, we made smears for *E. coli* from two groups respectively, and preformed gram staining\(^{[16]}\) to observe their sizes and morphology. Oil immersion lens observation showed that there was no significant difference in size and morphology of bacteria (Figure S9).

![Figure S9. Under the view of microscope, the cell size and morphology of *E. coli* before and after separation.](image)

After morphological observation, we tested the activity and function of the *E. coli* by bacterial culture and automatic microbiological biochemical analyzer (VITEK 2, bioMerieux Inc.). Diluted the sample collected from middle outlet 5×10^4 times, and took 0.5ml solution from each diluent for the inoculation on sterilized culture medium. Bacterial colonies cultured at 37°C for 24h as shown in Figure S10. The colony number of the control group and separated group were 186 and 164, and the concentrations of *E. coli* in the corresponding samples were 1.86×10^7/mL and 1.64×10^7/mL, respectively. Considering the target cell recovery rate of the system, cell culture results demonstrated that acoustofluidic sorting did not bring an unexpected

![Figure S10. the bacterial colonies of control group and separated group after 24h cell culture.](image)
effect on bacterial activity or function. In addition, *E. coli* from two groups were further qualitative analyzed by automatic microbiological biochemical analyzer, and the results demonstrated no difference in a series of biochemical test indexes. Therefore, this acoustofluidic device can maintain the activity and function of bacteria after separation.

**Broader prospects**

The method of increasing acoustofluidic throughput through serpentine structure is not limited to the device proposed in this paper. In addition to this design, where the pressure node lines are parallel to channel, we also applied the serpentine microchannel on taSSAW (tilted-angle standing surface acoustic wave)\(^5\)[\(^{17}\)] device and verified its enhancement on throughput by numerical simulation. The taSSAW device is characterized by a tilted angle \(\theta\) between IDTs, or pressure node lines in SSAW field, and microchannel (Figure S10.(a)). Driven by acoustic radiation force, particles in the SSAW field tend to focus on pressure nodes and gradually move towards one side along pressure node lines(Figure S10.(b)). The numerical simulation result of particle separation using the taSSAW device is illustrated in Video 4.

**Figure S11. Design of taSSAW device with a serpentine structure.** (a) Schematic of the acoustofluidic device. (b) Working mechanism of the acoustofluidic device.
Video 1. The numerical simulation of polystyrene particle separation in serpentine microchannel.

The simulated trajectories of 1 μm and 5 μm polystyrene microparticles in the acoustofluidic device while SAW is on.

Video 2. The trajectory of polystyrene particles (5 μm & 1 μm) in serpentine microchannel.

Real-time video of polystyrene particles in the acoustofluidic device while SAW is on and off.

Video 3. The trajectory of cells in serpentine microchannel

Real-time video of RBCs and E. coli in the acoustofluidic device while SAW is on and off. In the bright-field video, E. coli is not visible because of its small size and high throughput.

Video 4. The numerical simulation of polystyrene particle separation in taSSAW device with a serpentine structure.

The simulated trajectories of 1 μm and 5 μm polystyrene microparticles in the taSSAW device while SAW is on.

References
