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Particle separation in surface acoustic wave microfluidic devices using reprogrammable, pseudo-standing waves

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We report size and density/compressibility-based particle sorting using on-off quasi-stationary waves based on frequency difference between two ultrasonic transducers. The 13.3 MHz fundamental operating frequency of the surface acoustic wave microfluidic device allows manipulation of particles on the micrometer scale. Experiments, validated by computational fluid dynamics (CFD), were carried out to demonstrate size-based sorting of 5 to 14.5 µm diameter polystyrene (PS) particles; and density/compressibility-based sorting of 10 µm PS, iron-oxide and poly(methyl methacrylate) particles, with densities ranging from 1.05 to 1.5 g/cm³. The method shows a sorting efficiency of >90% and a purity of >80% for particle separation of 10 µm and 14.5 µm, demonstrating better performance than similar sorting methods recently published (72 to 83% efficiency). The sorting technique demonstrates high selectivity separation of particles, with the smallest particle ratio being of 1.33, compared to 2.5 in previous work. Density/compressibility-based sorting of polystyrene and iron-oxide particles showed 97±4% efficiency and 91±5% purity. By varying the sign of the acoustic excitation signal, continuous batch acoustic sorting of target particles to a desired outlet was demonstrated with good sorting stability against variations of the inflow rates.

Particle or living cell separation is a critical enabling step in industrial, chemical and biomedical processes.1 Acoustic separation techniques are especially advantageous for their non-contact, label-free, biocompatible properties.2 To achieve particles sorting, most acoustic separation methods utilize the difference in time-of-flight of particles subjected to a standing pressure wave, therefore limited to separation distances up to a quarter of the wavelength.3 Transducers tilted with respect to the axis of the separation channel alleviate the limited separation distance, but the translational distance of particles is still bound by the geometrical design.4

Particles can also be translated unconstrained by a quasi-standing wave, as a result of phase modulation or by small frequency difference between opposing transducers.5-7 In both techniques, the maximum translational speed of the particles has a size-dependence that can be utilized for sorting.8 Recently, a continuously frequency modulated acoustic fields method was applied in a surface acoustic wave (SAW) device.9 However, in this technique, the particle movement is fluctuating, demonstrating limited selectivity (2.5-fold or greater diameter ratio) and efficiency (up to 83%). We propose a modified method that increases both selectivity and efficiency to open up a wide range of applications in biomedical engineering and beyond.

Our previous works used phase modulation to achieve particle sorting.10,11 These were carried out in the absence of the flow, and sorting had been presented only in a single direction.
Moreover, phase modulation requires a more complex control signal than the frequency modulation technique presented in this letter.

In this letter, we extend the existing knowledge on particle separation using quasi standing waves and apply the method for particle separation in surface wave devices. Average particle speed measurements were carried out for various frequency differences, \( \Delta f \), ranging from -1.6 to 1.6 Hz. Within this interval the particles either linearly translate with the moving pressure node (below maximum particle velocity), or they are dominated by an oscillatory motion (above maximum speed). We provide a theoretical framework to allow adaptation of the design parameters to different geometries. This technique provides the possibility of steering target particles to different outlets by adjusting the flow rates and the sign of the modulation signal. We term these ‘upwards’ (target exit at top outlet) and ‘downwards’ (target exit at bottom outlet) sorting.

The schematic of the sorting device is shown in Fig. 1. The transducers have a central frequency of 13.3 MHz, corresponding to \( \lambda = 300 \, \mu\text{m} \) for a 128-Y cut X-oriented lithium niobate substrate. The polydimethylsiloxane (PDMS) channel has a width of 240 \( \mu\text{m} \), allowing for two acoustic pressure nodes. The desired symmetric pressure node distribution within the device can be achieved by changing the relative phase of the acoustic waves propagated by the transducers. More details on device fabrication and experimental setup have been presented elsewhere. The microfluidic device has an asymmetric inlet configuration: one sheath and sample inlet are 50 \( \mu\text{m} \) wide, while another sheath inlet is 140 \( \mu\text{m} \) wide. The flow rates at the three inlet channels are adjusted to focus the particles at one of the pressure nodes, termed the ‘focusing’ node (Fig. 1). When the frequency difference, \( \Delta f \), is created between the two transducers, the interplay between acoustic radiation and drag forces selectively displaces the larger particles towards the other pressure node, defined here as ‘sorting’ node. An asymmetric inlet configuration is designed to investigate how significant particle focusing is on the efficiency and purity on ‘upwards’ and ‘downwards’ sorting.

Two opposing transducers activated at the same frequency results in a standing wave field, where the acoustic radiation force collects particles at either the nodes or antinodes. When the two frequencies, \( f_1 \) and \( f_2 \), differ slightly, the resulting standing wave moves spatially with speed

\[
v_p = \frac{2\pi(f_1 - f_2)}{(k_{y,1} + k_{y,2})} = \frac{2\pi\Delta f}{(k_{y,1} + k_{y,2})}
\]

where \( k_{y,i} = 2\pi/\lambda_i \) with \( \lambda_i = c/f_i \), and \( c \) being the surface wave velocity, meaning the pressure nodes and therefore the particles are always displaced away from the higher frequency transducer. Assuming that \( \Delta f \ll f_0 \) and therefore \( k_{y,1} \approx k_{y,2} = 2\pi f_0/c \), where \( f_0 = 13.3 \, \text{MHz} \) is the central frequency of the transducer, and \( c \approx 3990 \, \text{ms}^{-1} \), the translational speed is

\[
v_p = 150 \cdot \Delta f \, \text{in \( \mu\text{m/s} \)}
\]
The acoustic radiation force can be obtained from the pressure distribution (see supplementary document)

\[ F_{ac,y} = -c_{ac} \sin(2k_y y - 2\pi \Delta f t) \]  

where all particle and medium dependent parameters are included in \( c_{ac} \), and \( k_y \approx 2\pi f_0/c \). As the particle is placed in a liquid medium, the Stokes’ drag force opposes the acoustic radiation force as a result of the inertial approximation: \(^{14}\)

\[ -F_{ac,y} = F_{\text{drag}} = -6\pi \eta R \dot{y} = -c_{\text{visc}} \dot{y} \]  

where \( R \) is the particle radius, \( \dot{y} \) is the relative speed of the particle with respect to the medium, and \( \eta \) is the dynamic viscosity of the medium. Wall effects of the channel can be incorporated into the viscosity. \(^{15}\) This equation can be used to obtain particle trajectories (see supplementary document).

Both the trajectory equation and the force balance predict a limit for the linear translation of particles. The maximum particle speed, is obtained from the maximum radiation force \(^{5}\)

\[ v_{\text{max}} = (\dot{y})_{\text{max}} = c_{ac}/c_{\text{visc}} \]  

Any frequency difference that causes a nodal translational speed \( v_p \) less than \( v_{\text{max}} \), forces the particles to move linearly with a constant speed. However, if the nodal speed is greater than the maximum speed \( v_p > v_{\text{max}} \), the particles oscillate and shift at the same time, in a less deterministic manner.

Speed measurements were carried out to demonstrate this phenomenon. Particle trajectories were recorded to calculate average particle speeds. Results for 19 Vpk-pk transducer voltage for 10 and 14.5 \( \mu \)m particles are shown in Fig. 2. For frequency differences between -0.4 and 0.4 Hz (Fig. 2, Region I), both particles are below their respective speed limit \( v_p < v_{\text{max}} \), so they both translate simultaneously, \(^{5}\) and no sorting can be achieved. When the frequency difference is less than -0.85 or greater than 0.85 Hz (Fig. 2, Regions III), as \( v_p > v_{\text{max}} \), both particles only oscillate with small average speeds, which cannot be used for sorting. However, in regions

![Fig. 2 (color online). Experimental and theoretical average particle speed for various frequency difference values between transducers. Insets are overlay images of the corresponding videos, illustrating particle motion.](image-url)
between -0.85 to -0.4 and 0.4 to 0.85 Hz (Fig. 2, Regions II), the large particles are below their maximum speed and can be translated linearly, while the small particles oscillate and shift with a lower average speed. These regions are promising for sorting applications. The regions from -0.8 to -0.4 Hz and from 0.4 to 0.8 Hz are defined as the downwards and upwards regimes when target particles exit via the lower and upper outlet, respectively. Although the frequency difference between transducers is six orders of magnitude smaller than the center frequency, speed measurements and separation experiments were highly reproducible showing good performance of the technique.

To utilize the sorting regions (Fig. 2, Regions II), we apply to both transducers the frequency pattern shown in Fig. 3a-b. The frequency difference, $\Delta f$, is switched on for a period of time $t_{ON}$, followed by an off period, $t_{OFF}$. Note that the transducers are on for the entire sorting process and only the frequency modulation switches periodically. The on period has a length of $t_{ON} = 1/\Delta f$, guaranteeing that the pressure nodes move half a wavelength, as illustrated in Fig. 3c. The off period allows the particles to reach an equilibrium position at the focusing node (small particles) or at the sorting node close to the target outlet (large particles) as shown in Fig. 3c. This on-off switching approach makes the sorting technique repeatable, as the oscillating small particles are forced to a fixed position periodically. We uploaded to the signal generator one period of the modulation pattern seen in Fig. 3a on the right and used internal frequency modulation, with modulation frequency $f_{mod} = 1/(t_{ON} + t_{OFF})$. Left column of Fig. 3 corresponds to upwards sorting, while the right column denotes downwards sorting.

COMSOL computational fluid dynamics was utilized to simulate the focusing of the particles at different positions within the PDMS microchannel. Details can be found in the supplementary document.
Particles of various sizes and densities/compressibilities were used experimentally. The sorting quality was assessed according to the following figures of merit:\(^{16}\)

\[
\text{efficiency} = \frac{\text{number of target particles in target region}}{\text{total number of target particles}}
\]  

(7)

\[
\text{purity} = \frac{\text{efficiency}}{\text{efficiency} + \text{non-target particles in target region to total number of non-target particles}}
\]

(8)

The fabricated microfluidic device presented an inhomogeneous pressure distribution along the SAW active area. The measured\(^{11}\) spatial variation in pressure was 30% higher at the sides of the active area than at the middle, where the average particle speed characterization experiments were carried out. Therefore, the frequency differences in the sorting experiments were increased by 40% compared to the values suggested by Fig. 2.

The experimental parameters and results are summarized in Table I. For the size-based sorting experiments, the particles were suspended in polyethylene glycol solution (PEG, 0.1% w/v in DI water) to avoid stiction of particles to sidewalls. Particle concentration was at least \(2 \cdot 10^6 \text{ mL}^{-1}\), and at least 100 particles were counted to have accurate efficiency and purity values. Five counting periods were randomly chosen and averaged within a 10 minute timeframe when the experiment was running. The voltage used in the experiments was 19-23 V\(_{pk-pk}\), lower values did not provide high enough acoustic force to reliably trap and manipulate the particles; higher values result in heat generation that is unfavorable for biological applications.

As detailed in the supplementary document, different sorting scenarios are equivalent when the particles to be separated have the same size ratio. For particle size ratio greater than 1.3, high efficiency and purity, both for the upwards and downwards sorting were recorded. In all these cases, the efficiency was higher than 84% and the purity higher than 81%. The efficiency for both upwards and downwards sorting drops to around 70% with the purity being approximately 75% when the particles size ratio decreased to 1.2. As 70-75% efficiency and purity can be treated as minimum desirable values, the limit of this separation method and device is therefore found to be size ratio of 1.2. Overlay images illustrating the sorting can be seen in Fig. 4.
Since the acoustic radiation force depends also on the particle density and compressibility, we carried out separation experiments for 10 µm particles of polystyrene, PS (\( \rho = 1.05 \text{ g/cm}^3 \), compressibility \( \kappa = 250 \text{ TPa}^{-1} \)), iron-oxide, FeO (\( \rho = 1.5 \text{ g/cm}^3 \), \( \kappa < 15 \text{ TPa}^{-1} \)) and poly(methyl methacrylate), PMMA (\( \rho = 1.2 \text{ g/cm}^3 \), \( \kappa = 170 \text{ TPa}^{-1} \)). To reduce sedimentation before entering the channel, these particles were suspended in 30% (w/v) iodixanol solution (from OptiPrep density gradient, Sigma-Aldrich, and DI water). The PS and iron-oxide particles showed excellent separability, as shown in Table I, with >97% efficiency and >91% purity for both sorting directions. With the reduced difference in density for the PMMA and iron-oxide particles, high efficiency and purity were achieved by using two different frequency modulations: for 1 Hz, high efficiency (>97%), and for 2 Hz, high purity (>86%) were measured. For this sorting scenario both figures of merit are lower, and their variation is higher for the upwards sorting. Our previous works also investigated sorting based on density and compressibility differences of particles. Although they showed higher efficiency values, they were carried out in the absence of flow. Therefore, no issues were present such as hydrodynamic focusing inaccuracies or the particles being subjected to the acoustic field for slightly different periods of time due to the parabolic flow profile and travel time though the device.

Similar acoustic methods achieving particle or cell separation of similar size or physical properties are listed in Table III. with the respective figures of merit. Most of the works only present a single efficiency value for characterizing the device, and therefore direct comparison with our method is difficult, since achieving high efficiency is possible even with extremely low purity. Nevertheless, in all cases our method shows superiority both in the figures of merit and particle size ratio.

<table>
<thead>
<tr>
<th>particle mixture</th>
<th>dir*</th>
<th>top in</th>
<th>middle in</th>
<th>bottom in</th>
<th>Voltage (V_p-k)</th>
<th>( \Delta f ) (Hz)</th>
<th>off time (s)</th>
<th>efficiency (%)</th>
<th>purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.5 and 10 µm PS</td>
<td>D</td>
<td>0.5</td>
<td>0.4</td>
<td>1.2</td>
<td>19</td>
<td>-1.3</td>
<td>2</td>
<td>94±2</td>
<td>87±4</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>1.4</td>
<td>0.3</td>
<td>0.3</td>
<td>19</td>
<td>1.4</td>
<td>4</td>
<td>94±2</td>
<td>81±6</td>
</tr>
<tr>
<td>8 and 6 µm PS</td>
<td>D</td>
<td>0.25</td>
<td>0.15</td>
<td>0.6</td>
<td>19</td>
<td>-1.2</td>
<td>3.5</td>
<td>85±4</td>
<td>83±5</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>0.9</td>
<td>0.16</td>
<td>0.2</td>
<td>19</td>
<td>1.15</td>
<td>4</td>
<td>84±4</td>
<td>81±7</td>
</tr>
<tr>
<td>5 and 6 µm PS</td>
<td>D</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
<td>23</td>
<td>-1.5</td>
<td>3</td>
<td>71±5</td>
<td>78±6</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>0.7</td>
<td>0.2</td>
<td>0.2</td>
<td>23</td>
<td>1.2</td>
<td>3</td>
<td>67±9</td>
<td>73±9</td>
</tr>
<tr>
<td>10 µm PS and FeO</td>
<td>D</td>
<td>1.1</td>
<td>0.4</td>
<td>0.3</td>
<td>23</td>
<td>-2.5</td>
<td>1.2</td>
<td>97±4</td>
<td>93±5</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>0.3</td>
<td>0.4</td>
<td>1.1</td>
<td>23</td>
<td>2.5</td>
<td>1.2</td>
<td>98±3</td>
<td>91±5</td>
</tr>
<tr>
<td>10 µm PMMA and FeO</td>
<td>D</td>
<td>1.3</td>
<td>0.2</td>
<td>0.5</td>
<td>19</td>
<td>-2</td>
<td>2</td>
<td>85±7</td>
<td>94±3</td>
</tr>
<tr>
<td></td>
<td>U</td>
<td>0.5</td>
<td>0.15</td>
<td>1.6</td>
<td>19</td>
<td>-2</td>
<td>2</td>
<td>91±8</td>
<td>86±10</td>
</tr>
</tbody>
</table>
Sensitivity analysis of the device was carried out by varying the flow rates at the various inlets for 10 and 14.5 µm diameter particles. The experiments were conducted with the same excitation signals reported in Table I. *Direction of sorting (Dir): upwards (U) or downwards (D). ‡Reference experiments, as shown in Table I.

<table>
<thead>
<tr>
<th>dir*</th>
<th>flow rates (µl/min)</th>
<th>efficiency (%)</th>
<th>purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>top inlet</td>
<td>middle inlet</td>
<td>bottom inlet</td>
</tr>
<tr>
<td>D‡</td>
<td>0.5</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>D</td>
<td>0.3</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>D</td>
<td>0.7</td>
<td>0.4</td>
<td>1.4</td>
</tr>
<tr>
<td>U‡</td>
<td>1.4</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>U</td>
<td>1.2</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>U</td>
<td>1.6</td>
<td>0.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

TABLE III. Comparison of the results with other acoustic sorting methods. E denotes efficiency, P denotes purity.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Device parameters</th>
<th>Particles or cells to be separated</th>
<th>Figure of merit</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON-OFF frequency switch (this work)</td>
<td>13.3 MHz, 50 µm channel height, 240 µm channel width</td>
<td>14.5 µm/10 µm PS, size ratio 1.45</td>
<td>E: 94±2%, P: 87±4%, 81±6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 µm PS and iron-oxide</td>
<td>E: &lt;98±3%, P: 93±5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 µm PMMA and FeO</td>
<td>E: &lt;99±1%, P: 94±3%</td>
</tr>
<tr>
<td>Continuously phase modulated by frequency step</td>
<td>14 MHz, 1050 µm channel width, 80 µm height</td>
<td>15 µm/6 µm PS, size ratio 2.5</td>
<td>E: 72%, P: n/a</td>
</tr>
<tr>
<td>Continuing wave sorter</td>
<td>14 MHz, 1050 µm channel width, 80 µm height</td>
<td>2 µm PS particles, HaCaT cells (mean dia 24.47 µm), size ratio &gt;10</td>
<td>E: 83%, P: n/a</td>
</tr>
<tr>
<td>Standing wave sorter</td>
<td>13.2 MHz, 300 µm width, 100 µm height</td>
<td>3 and 10 µm PS, size ratio 3.33; 3 and 5 PS, size ratio 1.67</td>
<td>E: for 3 µm 87.4–94.8%; for 10 µm 94.6–100%, no data for 3/5</td>
</tr>
<tr>
<td>Standing wave sorter</td>
<td>13.5 MHz, 150 µm width, 80 µm height</td>
<td>10 µm PS (1.05 g/cm³) and melamine (1.71 g/cm³)</td>
<td>E: 87.2–98.8%, P: n/a</td>
</tr>
</tbody>
</table>

Sensitivity analysis of the device was carried out by varying the flow rates at the various inlets for 10 and 14.5 µm diameter particles. As a reference, we used flow rates corresponding to the best performance sorting scenario, and varied the sheath inflows, from 0.2 µl/min less than the reference to 0.2 µl/min above the reference. When examining only the trapping performance of the device, at least 97% of the particles were trapped towards the non-sorting outlet. The 3% particle loss was observed for low sheath or high particle flow, and was due to acoustic streaming at the channel walls, trapping particles towards the walls. Sorting experiments for various flow rates are listed in Table II. When the sheath flow on the non-target outlet side was small, particles attached to the sidewall due to acoustic streaming. Interestingly, even for increased sheath flows, the efficiency dropped. We attribute this decrease in efficiency to the faster transport of particles and therefore not enough time spent in channel for sorting. Upwards sorting was more susceptible to variations, which suggests that the asymmetric inlet design favors the more natural focusing at the top node and sorting towards the bottom outlet.
In conclusion, we presented an easily reconfigurable method for particle separation in surface wave microfluidic devices increasing the performance of acoustic sorting techniques that are based on frequency difference between two transducers. Bidirectional sorting has been demonstrated by adjusting the inflow rates and electrical signal of transducers. The separation distance achieved with this method is half the wavelength, which is double as that of conventional time-of-flight methods. This technique can be applied for various particle separation scenarios due to its versatility, reconfigurability and simple electrical excitation requirements. This technique can also be scaled easily, and adjusting the frequency is straightforward given the scaling laws in the supplementary document. Future work will analyze the application of the method for biological living cell separation.

See supplementary material for details on derivation of acoustic radiation force in quasi-standing waves, simulation of velocity profile and particle focusing within the microchannel, and scaling of optimal sorting frequency range for various experimental parameters.

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14H. Braus, Lab Chip 12, 1578 (2012).
waste
sorted particles
narrow sheath
sample
focusing node
wide sheath
sorting node
focusing node
waste
sorted particles
f
1
f
2
x
y
z
50 µm
240 µm
140 µm
120 µm
10 mm
6 mm
120 µm
10 mm
120 µm
50 µm
(a) Upwards sorting

\[ f_2 \]
\[ f_0 + \Delta f \]
\[ f_0 \]

(b) Downwards sorting

\[ f_1 \]
\[ f_0 + \Delta f \]
\[ f_0 \]

(c) Position

\[ \lambda/4 \]
\[ -\lambda/4 \]

(II) (III)
Upwards sorting

Downwards sorting

Size separation

Density-compressibility separation

(a)

(b)

(c)

(d)