Mechanically robust microfluidics and bulk wave acoustics to sort microparticles

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ABSTRACT

Sorting microparticles (or cells, or bacteria) is significant for scientific, medical and industrial purposes. Research groups have used lithium niobate SAW devices to produce standing waves, and then to align microparticles at the node lines in polydimethylsiloxane (PDMS, silicone) microfluidic channels. The “tilted angle” (skewed) configuration is a recent breakthrough producing particle trajectories that cross multiple node lines, making it practical to sort particles. However, lithium niobate wafers and PDMS microfluidic channels are not mechanically robust. We demonstrate “tilted angle” microparticle sorting in novel devices that are robust, rapidly prototyped, and manufacturable. We form our microfluidic system in a rigid polymethyl methacrylate (PMMA, acrylic) prism, sandwiched by lead-zirconium-titanate (PZT) wafers, operating in through-thickness mode with inertial backing, that produce standing bulk waves. The overall configuration is compact and mechanically robust, and actuating PZT wafers in through-thickness mode is highly efficient. Moving to this novel configuration introduced new acoustics questions involving internal reflections, but we show experimental images confirming the intended nodal geometry. Microparticles in “tilted angle” devices display undulating trajectories, where deviation from the straight path increases with particle diameter and with excitation voltage to create the mechanism by which particles are sorted. We show a simplified analytical model by which a “phase space” is constructed to characterize effective particle sorting, and we compare our experimental data to the predictions from that simplified model; precise correlation is not expected and is not observed, but the important physical trends from the model are paralleled in the measured particle trajectories.

Keywords: Bulk waves, microfluidics, microparticles, PMMA, standing waves

1. INTRODUCTION

Separating and sorting cells or bacteria is an important challenge, and this work is situated within engineering research activities that combine microfluidics and ultrasonics to do so. The particular research approach uses ultrasonic standing waves to create acoustic pressure fields in the microfluidic channel; specifically, nodes (or antinodes) form in parallel lines to which the microparticles move, and separation is achieved by a suitable combination of microfluidic behavior and ultrasonic performance. Particles of interest are in the size range of 1 – 20 µm, with node line spacing on the order of 100 µm. Prominent research groups typically use soft lithography in polydimethylsiloxane (PDMS, more commonly known as silicone) to fabricate the microfluidic device, mounted on lithium niobate wafers patterned to perform as SAW devices generating standing surface acoustic waves (SSAW).

These research activities are correctly described as “lab-on-chip.” However, an accompanying research challenge can be paraphrased as “a chip in a lab is not truly a lab on a chip.” In this paper we report on our success with two major contributions to the microparticle sorting activity. We first show a geometric configuration of the combined devices, termed the “tilted angle” configuration, providing developers with suitable control of design and operating parameters to achieve practical particle sorting. We next show a mechanically robust configuration in which the microfluidic device is fabricated in a polymethyl methacrylate (PMMA, more commonly known as acrylic or plexiglass) prism, sandwiched between piezoceramic elements (lead zirconium titanate, PZT) that produce bulk waves to generate the standing waves. Our PMMA rigid prism configuration is a major step in moving this lab-on-chip technology out of the laboratory because it is mechanically robust and manufacturable. Moreover, it is rapidly prototyped; we show results with devices in which a new channel geometry and/or transducer type was ideated, fabricated, instrumented, and tested within hours.

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2. PRIOR WORK

Important work by other research groups predates our involvement with these combined microfluidic/ultrasonic devices, exemplified by influential work from Huang\(^1\) and his collaborators at Penn State and from Johannson\(^2\) and her collaborators in Stockholm. Figures 1 through 3 reproduce images from our earlier publications\(^3, 4, 5, 6\) showing a PDMS microfluidic channel mounted on a lithium niobate wafer that generates standing surface acoustic waves (SSAW). That device illustrates dimensions and operational conditions that are representative of the field, unless noted otherwise, and are summarized as follows:

- Figure 1 shows a SAW device patterned on a lithium niobate wafer of 0.5 mm thickness. A pair of opposed interdigitated transducer (IDTs) generate surface acoustic waves; they are driven by a steady-state excitation, in their opposed configuration, to produce the standing wave condition.
- Each IDT consists of 20 finger pairs with a feature size (finger width and clear spacing) of 50 \(\mu\)m, corresponding to a wavelength of 200 \(\mu\)m. The accompanying spacing between node lines is 100 \(\mu\)m.
- (The resonant frequency is nominally 20 MHz for a device fabricated on 127.8° rotated Y-cut lithium niobate.)
- The PDMS microfluidic device contains a channel that is 0.5-mm square in cross-section; those dimensions are not typical of the field, because we cast our devices with a mechanical (rather than lithographic) mold, but those particular dimensions do not otherwise govern or strongly influence the behavior described here.
- Figure 1 shows the typical configuration, in which the channel is nominally parallel to the IDTs; accordingly, node lines will be parallel to the channel walls and parallel to the direction of flow (the streamlines).

![Figure 1](image1.png)

Figure 1. Surface acoustic wave (SAW) device consisting of opposing interdigitated transducers (IDTs) fabricated on a lithium niobate wafer, with a microfluidic channel formed in polydimethylsiloxane (PDMS)\(^4\).

- Figure 2 (top) is a microscope image of 6 \(\mu\)m polystyrene particles, randomly distributed in the channel.
- Figure 2 (bottom) is a microscope image of the particle distribution after driving the IDTs at 4.3 V for 30 sec, showing concentration of particles at node lines.
- As noted above, the node line spacing observed in Figure 2 (bottom) is approximately 100 \(\mu\)m.
- Figure 2 was obtained for the simplifying case in which no flow was induced. In operation, a syringe pump produces a controlled volumetric flow rate, but initial observations of particle movement to nodes are more clearly made in the no-flow case.
Particles of different size travel to the nearest node line at different speeds, as plotted from our measurements in Figure 3. That difference in approach speed was intended to create a separation mechanism, using carefully spaced branches to collect particles selectively. However, the customary configuration, in which standing wave node lines form parallel to the flow streamlines, limits the particle “deflection” (referring to particle displacement transverse to the direction of flow) to the spacing between node lines.

That inherent geometric constraint represented a considerable engineering challenge for effective separation. That challenge was overcome by an alternate configuration in which the microfluidic channel would be placed not parallel to the IDTs, but instead as some oblique, skewed, or “tilted angle” configuration. That configuration was developed independently in our lab and by a prominent research group (including Dao, Suresh, and Huang) that has also demonstrated the separation of tumor cells from blood cells. The tilted angle configuration was demonstrated for a PDMS microfluidic device combined with a SAW device (IDTs patterned on a lithium niobate wafer) that was not otherwise different from earlier work, but we introduced the alternative of microfluidic device fabrication in PMMA.

Representative results of our earlier work are shown in Figures 4 through 6, described below:

- Figure 4 shows an example from our initial study of a microfluidic device forming a channel at a “tilted angle” with respect to the node lines (and the IDT fingers) of a SAW device.
- The underlying SAW device, referring to the opposed IDTs patterned on a lithium niobate wafer, is not significantly different from the device described earlier in Figure 1.
The microfluidic device contains a channel oriented skew to the IDTs; we studied various skew angles between 10° and 30°.

The microfluidic device pictured in Figure 4 was fabricated (micromachined) in PMMA, but is otherwise geometrically similar to the microfluidic devices fabricated in PDMS.

Figure 4. Microfluidic device housing a channel at a tilted angle with respect to node lines.

Microparticles in a tilted angle channel can follow “undulating paths,” in which they can deflect (move transversely from the flow streamline) in a stepping motion past multiple node lines. The undulating path, and the mechanism whereby paths for different-sized particles can be controlled by the user, results from the action on a particle of a fluid (drag) force acting in the direction of the flow streamline, and an acoustic (pressure-induced) force acting normal to the skew angle. Those forces scale differently with particle size; large particles experience stronger acoustic forces than small particles. The reader should also envision an operator controlling the flow velocity (to vary the fluid force) in combination with the IDT voltage (to vary the acoustic force) in order to produce an intended trajectory for particles of interest.

Figure 5. Simulated particle paths (white, blue, yellow, magenta, cyan, red, and green; correspond to 3, 4, 5, 6, 7, 8, 9 µm diameter) in tilted angle configuration, showing characteristic undulations.

Figure 5 plots simulation results showing particle movements at a particular combination of flow velocity and acoustic pressure, qualitatively demonstrating the behavior mechanism by comparing the paths followed by particles of different sizes. The pressure is plotted as a grey-scale color (such that the parallel dark lines are node lines) and the fluid velocity is horizontal to the left. Particles are introduced at one point with an initial velocity parallel to the node line. The smallest particles (the white and blue paths, 3 µm and 4 µm diameter particles) travel horizontally to the left almost immediately because the fluid force is much greater than the acoustic force. (The inertial force is smaller than either the fluid force or the acoustic force, and is ignored in this simulation.) The largest particle (the green path, a 9 µm diameter particle) follows a path that is substantially influenced by the acoustic force, but which steps from one node line to another in response to the fluid force. Particles between those sizes follow undulating paths with a shorter travel distance along a node line (which may be termed a “tread” dimension) before being deflected to the next node line.
Figure 6 is a previously published image tracing segments of paths followed by two particles of different size, 4.5 µm and 15 µm diameter. (The horizontal lines in that image are artifacts of the imaging process, comparable to raster scan lines, and the reader should ignore them.) The red trace, a small particle, is not significantly influenced by the acoustic force, and we attribute its deviation from linear to local variations in the flow condition. The blue trace, a large particle, is strongly influenced by the acoustic force and follows the characteristic undulating path; three “tread” segments along node lines (bearing roughly 15° south of west) and two intervening “rise” segments are captured in the image.

### 3. THE PMMA RIGID PRISM CHANNEL AND ULTRASONIC BULK WAVES

We fabricate microfluidic channels in PMMA by milling at sub-mm scale on a Mini-Mill desktop CNC system from Minitech Machinery Corp. Our first PMMA microfluidic structure is the channel, mounted on a SAW device, shown in Figure 4. We now report our fabrication of a tilted angle channel internal to a mechanically robust PMMA prism\(^ {10,11}\).

We fabricate PMMA prisms from two half-pieces that join to form a prism with an internal channel. Each half-piece is manufactured from PMMA stock 3.175-mm thick. One half-piece is milled to create the microfluidic channel, and both half-pieces are milled to form inlet/outlet segments, alignment holes, and transducer through-slots. We bond the two half-pieces by cleaning first with soap and water, ashing the faces to be bonded in a Harrick Plasma Cleaner (in air) for five minutes, and then clamping the two pieces between glass plates and baking in an oven at 305°F for 60 minutes.

Figure 7 shows two completed devices, consisting of the PMMA prism containing the internal microfluidic channel, sandwiched between two PZT slabs, with inertial backing, operating in through-thickness mode to generate bulk waves. Figures 7 (left) shows a first device, whereas Figure 7 (right) shows a later device containing numerous engineering improvements, outlined below:
• The fluid inlets and outlet in the first device enter normal to the plane containing the microfluidic channel, while those structures are co-planar (or co-axial) in the later device. The (average) fluid velocity is typically between 0.25 and 2.5 mm/sec.

• The later device uses three inlets, a central (axial) inlet for the fluid containing particles and two neighboring inlets for water, from which the flow meets in a trident geometry to produce hydrodynamic focusing of particles as imaged in Figure 8.

• The later device is fabricated with alignment keys to achieve “snap-together” assembly with sufficient precision to create complex microfluidic channels and to present a planar face for uniform acoustic coupling.

• The PZT slabs, with a tungsten rubber backing slab, were glued to the prism side faces in the first device. However, in the second device, those components are contained within slots and coupled acoustically to the PMMA prism with water, way oil, or acoustic coupling gel. We drive the transducers at roughly 6.6 MHz.

• The fabrication process permits very rapid prototyping. Design improvements from the first device to the later device, as shown in Figure 7, required fabricating roughly 50 different configurations but was accomplished in less than six months. Of course, the rapid prototyping capability is even more valuable for enabling the testing of different device configurations to address new research questions and to develop devices for performance in particular particle-sorting and cell-sorting applications.

Most significantly, the device in Figure 7 (right), combining a PMMA prism with transducers that produce standing ultrasonic bulk waves, is mechanically robust and manufacturable.

Figure 8. Hydrodynamic focusing for a mix of 2µm and 15 µm particles. (This image is a particle trace of 4 sec duration.)

4. PARTICLE TRAJECTORIES

Our raw experimental results are videos recorded with a monochrome camera through a microscope. Figures 9 through 11 show representative results11 as particle trace images that we create by plotting the minimum value of each pixel in the field. Figure 9 shows 15 µm diameter particles following trajectories that separate those larger particles from 2 µm particles. We observe other trajectories by which 15 µm and 2 µm particles separate in Figure 10, showing a device with somewhat different channel geometry, at a different combination of water velocity and acoustic pressure.
Figure 9. Separation of 2 \( \mu \)m and 15 \( \mu \)m particles

Figure 10. Separation of 2 \( \mu \)m and 15 \( \mu \)m particles; the 15 \( \mu \)m particles follow an undulating path while the 2 \( \mu \)m particles do not deflect from the streamlines of the microfluidic flow.

Figure 11 shows an attempt to separate 4.5 \( \mu \)m and 15 \( \mu \)m particles in which two 15 \( \mu \)m particles form a slow-moving paired particle. At the acoustic pressure used in this test, the paired particle follows an undulating path while 15 \( \mu \)m particles show partial deflection and 4.5 \( \mu \)m particles show no deflection from the streamlines of the microfluidic flow.

Figure 11. Separation of a large (paired) particle from 4.5 \( \mu \)m and 15 \( \mu \)m particles.

5. MAPPING THE DESIGN (AND OPERATION) SPACE FOR PARTICLE SEPARATION

We developed approximate models to simulate particle paths\(^{10, 11}\) and here we describe insights into particle separation that we have generated using those results. Consider the question of separating particles of two different sizes using a device for which the channel angle and channel length has been set. Two important parameters, the acoustic pressure and the water velocity, remain available to the user to achieve robust separation. We use the approximate models for particle paths to map different trajectories and to map the conditions under which the differently-sized particles can be separated. The resulting map can be considered a design space, or operation space, from which to choose good parameters. We discuss and illustrate these results to separate 15 \( \mu \)m particles from 2 \( \mu \)m particles, in a channel 4 mm long and 1.27 mm wide, over which standing waves form at a tilted angle of 30°.
We start by considering a particle to follow one of three distinct trajectories: (1) the particle follows the fluid streamline with no “deflection” by acoustic effects; (2) the particle follows an undulating path; (3) the particle follows a path to (“deflects to”) a node line. The boundary between trajectories (2) and (3) is the parameter pair making the deflection angle equal to the channel tilt angle. The boundary between trajectories (1) and (2) is the parameter pair producing minimal deflection, which we define as a deflection no greater than the particle radius over the length of the channel. On a plot of acoustic pressure against water velocity, these two boundaries plot as parabolas. (For a given transducer material type and thickness, acoustic pressure can be calculated as a linear function of applied voltage.)

![Figure 12. Modeled separation behavior (regions I through IV) pertaining to a mixture of 2 µm and 15 µm particles; see text for definition of particle behavior in each region.](image)

Plotting acoustic pressure against water velocity, if we plot the two boundaries, described above, for each of the two particle sizes that we seek to separate, in this example 15 µm and 2 µm, we obtain four parabolas that divide the design space into five regions. That plot is shown in Figure 12, in which four regions (labeled I through IV) are of interest. The conditions in those four regions are described physically as follows:

- In region I, the acoustic force on a particle is sufficiently large, relative to the fluid force, that 15 µm and 2 µm particles all deflect to a node line, and separation does not occur.
- In region II, 15 µm particles deflect to a node line while 2 µm particles follow an undulating path, because the trajectory of a large particle is more sensitive to acoustic pressure than that of a small particle. Separation is possible, in principle, but is not maximally effective.
- In region III, 15 µm particles deflect to a node line while 2 µm particles do not deflect. This achieves maximal, robust separation.
- In region IV, 15 µm particles follow an undulating path 2 µm particles do not deflect. Separation is possible, in principle, but not maximally effective.
- (Region V, not labeled, is the area on the abscissa of low acoustic pressure in which neither particle deflects.)

In this example case, 15 µm and 2 µm particles are very different in size. This makes those particles relatively easy to separate, as reflected by the large area occupied by region III in Figure 12, and by the wide range of operating parameters that can combine to place the system in region III and produce robust particle separation. Those same steps (plotting the boundaries between regions) can be used to visualize the limits of robust separation, such as the particlesize difference at which region III does not form.

6. CONCLUSIONS

We show two recent advances in the combination of microfluidics and ultrasonic acoustics to achieve sorting of microparticles and cells. The first advance is the tilted-angle configuration, which enables sorting that is functionally robust by separating particles through relatively large distances. We show laboratory results illustrating that concept, and we show modeling results that form a space capturing key relationships between design (and operation) parameters and effective separation performance. The second advance is the PMMA rigid prism, containing internal microfluidic...
channels, with standing ultrasonic bulk waves generated by PZT slabs. This device is more mechanically robust, more manufacturable, and more rapidly prototyped than our earlier configurations involving a PDMS structure mounted on a SAW device patterned on a lithium niobate wafer. In our opinion, the new configuration using a PMMA rigid prism and bulk wave acoustics helps move this lab-on-chip away from the confines of the laboratory.

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