MICROFLUIDIC PARTICLE MANIPULATION

The present invention relates to the manipulation and, more particularly, to the sorting of particles. Still more particularly, the present invention relates to the use of acoustic waves, including surface acoustic waves, for the manipulation and sorting of particles. In an example, the present invention may be used for the sorting of cells.

Rapid cell and particle sorting is an essential process in many research, industrial and clinical medicine applications, including cancer research,[1,2] reproductive technologies,[3] directed evolution,[4] high-throughput drug screening,[5] production of acoustic contrast agents,[6] water monitoring[7] and rare cell enrichment.[8,9] To discriminate between cell populations, these methods ideally make use of a physical parameter that differs between different populations, such as size,[10-12] magnetic susceptibility,[13-15] acoustic contrast,[16,17] electrical permittivity[18] or deformability,[19] so that sorting occurs passively in the presence of a continuously applied force field and/or flow. In the case of cells, this methodology can be readily used to sort between different types of cells, though is less adept at sorting within heterogeneous cell populations, where fluorescence detection[20] or impedance spectroscopy[21,22] can discriminate based on parameters such as cell function or morphology. Though more versatile, these methods require a separate and coordinated on-demand actuation system to physically translate the selected cells to a separate reservoir. Fluorescence activated cell sorting (FACS) in flow cytometry is the gold standard for cell sorting, where selected cells are electrically charged, aerosolized and subsequently deflected in an electric field.[23] Though FACS is used to sort at high rates, the aggregate systems are typically large, expensive, and not amenable to portable platforms.

There is increasing interest in equivalent on-chip sorting systems, many of which aim to replicate the sorting efficacy of that in conventional flow cytometry in an inexpensive integrated platform, though this has necessitated the development of microscale actuation systems that do not require the generation of a cell-containing aerosol as is the case in FACS. Most commonly, dielectrophoretic forces are applied locally to droplets containing cells due to large permittivity differences between oil and water phases,[24] where droplets are a useful tool for locally containing the volume surrounding cells,[25] though is throughput-

limited in the case of sorting in a single phase. Alternatively, Chen *et al.* demonstrated a device utilizing pulsed-laser induced cavitation to translate individual cells to a separate outlet stream at kHz rates.[26] However, the biocompatibility of cavitation in this context is not well understood, considering that cavitation has been used elsewhere for cell lysis.[27]

Acoustic fields present an attractive biocompatible alternative for sorting, where cells have been trapped while retaining their viability/morphology over periods of exposure from minutes to hours,[28-33] and where electrical driving circuits can be readily miniaturized for portable and cost-effective use.[34] Utilizing bulk acoustic waves (BAW) generated by a bulk wavemode transducer affixed to a resonant chamber, Jakobsson *et al.* demonstrated the active sorting of fluorescent beads in a continuous flow, where select particles were translated to the standing wave nodal position.[35] Surface acoustic wave (SAW) transducers, however, are generally easier to integrate with microfluidic systems, where the shape of the acoustic field can be easily defined by choosing an appropriate interdigital transducer (IDT) pattern and location, with IDTs defining the SAW path on a piezoelectric substrate. SAW has similarly been applied to high-throughput sorting; Nawaz *et al.* and Ren *et al.* used opposing IDT structures to generate sub-ms standing SAW pulses, where selected particles and cells are translated as they pass through the acoustic field,[36,37] conceptually similar to work by Ding *et al.*, where frequency shifts in the standing wave field resulted in shifts in the nodal position.[38]

As applied, however, there is room to improve on the robustness and selectivity of standing SAW-based sorting, where finely tuned inlet and outlet flow rates are required because particles are translated small distances. This is an inherent feature of standing waves, where the maximum displacement is less than one quarter of the acoustic wavelength. Thus, the smallest wavelength that can be used is dictated by the translation distance required. However, as the maximum translation distance is proportional to the acoustic wavelength in the case of a standing SAW, and as the minimum beam width is on the order of the acoustic wavelength, there is a trade-off between the particle displacement and the width of the sorting region. The minimum translations required for the sorting of cells using standing SAW, on the order of 20 μm, thus generate sorting regions with a width of ~150-300 μm.[36,37]

Travelling waves have a distinct advantage for sorting applications in that there is no inherent limit in the distance by which a particle subject to the resulting force field can be displaced, though the field strength will decay exponentially in the propagation direction, where travelling SAW has been used extensively for size-based separation and fluid manipulation over distances greater than λ.[39-45] Franke et al. and Schmid et al. used travelling SAW to rapidly sort particles, droplets and cells, where the acoustic field is localized using a circular polydimethylsiloxane (PDMS) post connecting the SAW substrate to a flow channel and actuated with an IDT structure that permits beam location adjustment.[46,47] The displacement is also limited in this case, however, where the acoustic wave attenuates as it passes through the post coupling the substrate wave to the channel and will only occur above the post.[48] The minimum width permitted is also limited practically by non-trivial fabrication constraints and the need to use a post that is at least on the same scale as λ, where larger λ are needed to minimize efficiency-reducing attenuation through the post structure. Phononic structures have the potential to direct acoustic energy to specified regions,[49-53] but typically require the use of a coupling material that reduces device efficiency,[54] and has not yet been applied to rapid sorting.

Avoiding the use of additional coupling structures to concentrate or localize acoustic energy, highly focused high-frequency SAW has substantial potential in microfluidic applications, where acoustic field gradients can be generated on the scale of 10's of μm. Previous work has demonstrated the potential for focused travelling SAW to concentrate energy at a particular location for efficient and targeted actuation, including for droplet translocation, droplet production and particle concentration.[55-58]

As such, typically, existing techniques all suffer from slow response times and consequently low sorting rates or low cell viability under high electric fields. Accordingly, improvements in cell sorting devices and methods are needed.

The listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

Any document referred to herein is hereby incorporated by reference in its entirety.

The present invention relates to an improved method and device for particle sorting, manipulation, and characterization that would be useful for numerous applications. In an embodiment, the present invention includes the concentration, focusing, and/or characterisation of particles such as cells and/or microorganisms.

In a first aspect of the present invention, there is provided a microfluidic device for manipulating a particle in a fluid suspension, the device comprising: (a) a substrate; (b) a channel defined in the substrate, the channel having an inlet for receiving the fluid suspension and an outlet for discharging the fluid suspension; and (c) an acoustic source configured to deliver a travelling surface acoustic wave transverse the flow of the fluid suspension in the channel, wherein the acoustic source is an interdigital transducer (IDT), the IDT comprises a plurality of concentric circular arcs having a tapered end directed at the channel, and the tapered end has an aperture of between 4 μm and 150 μm.

Any suitable technique may be used to create the travelling surface acoustic wave. For example, the surface acoustic wave may be created by a generator attached to the surface of the microfluidic channel. In certain embodiments, the surface acoustic wave is created by using an interdigitated electrode or transducer able to convert electrical signals into acoustic wave able to travel along the surface of the substrate, and in some cases, the frequency of the surface acoustic wave may be controlled by controlling the spacing of the finger repeat distance of the interdigitated electrode or transducer.

By "interdigital transducer (IDT)", it is meant to refer to any one, two, or more electrodes containing a plurality of "fingers" extending away from the electrode, wherein at least some of the fingers are interdigitated. In an embodiment, particularly when the piezoelectric substate is lithium niobate, there are about 20 to 100 finger pairs in the electrode. The fingers may be of any length, and may independently have the same or different lengths. The fingers may be spaced on the transducer regularly or irregularly. In some cases, the fingers may be substantially parallel, although in other embodiments they need not be substantially parallel. In an embodiment, the present IDT comprises a plurality of concentric

circular arcs having a tapered end that is directed towards the microfluidic channel. The tapered end forms the beam aperture. The angle subtended at the center of the concentric circular arcs of the IDT may be about 5 to 90 degrees. Preferably, in an embodiment, the angle is about 26 degrees.

The IDT may be supplied with any suitable AC supply known to the skilled person. The acoustic energy is maximized at the resonance frequency that is determined by the dimensions of the electrode fingers, size and spacing.

In an embodiment, the aperture size of the tapered end of the IDT is 56 μm. Advantageously, the size of the aperture produces a focused beam that allows the present microfluidic device to implement single-cell sorting. By "aperture", it is meant to refer to the width of the inner arc of the IDT that is directed towards the microfluidic channel. The 56 μm wide aperture can produce a 25 μm wide travelling sound beam giving rise to a 25 μm wide sorting/manipulation region within the microfluidic channel. The aperture size can be one wavelength to 15X wavelength, more preferably, the aperture size is about from 4-150 μm. In this embodiment, 10 μm is the wavelength, and the 56 μm aperture is 5.6X wavelength.

Preferably, the substrate is a piezoelectric substrate. More preferably, the piezoelectric substrate is lithium niobate. Alternatively, any suitable piezoelectric material may be used for the substrate. For example, lithium tantalite, lanthanum gallium silicate or the like.

The surface acoustic waves can be formed on a piezoelectric substrate or other material that may be coupled to a microfluidic substrate at specific locations, e.g., at locations within the microfluidic substrate where sorting is to take place. Suitable voltages (e.g., sinusoidal or other periodically varying voltages) are applied to the piezoelectric substrate, which converts the electrical signals into mechanical vibrations, i.e., surface acoustic waves or sound. The sound is then coupled to the microfluidic substrate, e.g., from the surface of the material. In the microfluidic substrate, the vibrations pass into liquid within microfluidic channels in the microfluidic substrate, which give rise to internal streaming within the fluid and acoustic radiation that directly exerts a force on particles exposed to the acoustic field.

Thus, by controlling the applied voltage, streaming within the microfluidic channel, as well as the acoustic radiation force on particles, may be controlled, which may be used to direct or sort particles within the microfluidic channel, e.g., to particular regions within the microfluidic substrate. The travelling surface acoustic wave may induce two acoustic effects – an acoustic streaming and an acoustic radiation. In addition to acoustic radiation, acoustic streaming may also be dominant if a suitable power is supplied to the IDT in the microfluidic device. Advantageously, acoustic streaming may also provide useful cell manipulation capability to the microfluidic device.

Advantageously, the present invention requires only one IDT to generate the travelling the surface acoustic wave placed adjacent the microfluidic channel to deliver the wave transverse the flow of the fluid suspension in the channel.

The piezoelectric substrate may be activated by any suitable electronic input signal or voltage to the piezoelectric substrate (or portion thereof). For example, the input signal may be one in which a periodically varying signal is used, e.g., to create corresponding acoustic waves. For instance, the signals may be sine waves, square waves, sawtooth waves, triangular waves, or the like. The frequency may be for example, between about 50 Hz and about 100 KHz, between about 100 Hz and about 2 kHz, between about 100 Hz and about 1,000 Hz, between about 1,000 Hz and about 10,000 Hz, between about 10,000 Hz and about 100,000 Hz, or the like, and/or combinations thereof. In some cases, the frequency may be at least about 50 Hz, at least about 100 Hz, at least about 300 Hz, at least about 1,000 Hz, at least about 3,000 Hz, at least about 10,000 Hz, at least about 30,000 Hz, at least about 100,000 Hz, at least about 300,000 Hz, at least about 1 MHz, at least about 3 MHz, at least about 10 MHz, at least about 30 MHz, at least about 100 MHz, at least about 300 MHz, or at least about 1 GHz or more in some embodiments. In certain instances, the frequency may be no more than about 1 GHz, no more than about 300 MHz, no more than about 100 MHz, no more than about 30 MHz, no more than about 10 MHz, no more than about 3 MHz, no more than about 1 MHz, no more than about 300,000 Hz, no more than about 100,000 Hz, no more than about 30,000 Hz, no more than about 10,000 Hz, no more than about 3,000 Hz, no more than about 1,000 Hz, no more than about 300 Hz, no more than about 100 Hz, or the like. In a preferred embodiment, the frequency of the travelling surface

acoustic wave is greater than 50 MHz and, more preferably, about 386 MHz. It is discovered that, with the present invention, a higher frequency results in the generation of a highly focused and confined beam which, in turn, results in a force allowing the manipulation of the particles.

The IDT may be positioned on the piezoelectric substrate (or other suitable material) such that acoustic waves produced by the IDT are directed at a region of acoustic coupling between the piezoelectric substrate and the microfluidic channel. This region may also be referred to as the sorting/manipulation region.

In an embodiment, The IDT and the microfluidic channel shares the same substrate. The IDT is fabricated by depositing metallic electrodes on the piezoelectric substrate; the microfluidic channel may be an open layer with concave channel. By bonding this open layer onto the piezoelectric substrate, it can close the open channel and form a closed microfluidic channel. As the beam does not have uniform width along the wave propagation. So the distance from the IDT to the channel is also critical to ensure a highly confined sound beam in the channel region. The distance from the inner arc (tapered end) of the IDT to the microfluidic channel is about 0-1.5 mm. In the embodiment with an IDT aperture of 56 μm as described earlier, the minimum beam width of the wave is about 25 μm at the position that is 0.3 mm from the IDT.

Alternatively, the piezoelectric substrate and the microfluidic channel may be coupled or physically bonded to each other, for example, using ozone plasma treatment, or other suitable techniques. In some cases, the rest of the piezoelectric substrate and the microfluidic substrate are at least acoustically isolated from each other, and in certain embodiments, the piezoelectric substrate and the microfluidic substrate are physically isolated from each other. Without wishing to be bound by any theory, it is believed that due to the isolation, acoustic waves created by the interdigitated electrode and the piezoelectric substrate do not affect the microfluidic substrate except at regions where sorting is generally desired, e.g., at one or more coupling regions. Such acoustic coupling may be used, in certain embodiments, to increase sorting speed, e.g., due to better control of the passage of surface acoustic waves.

In one set of embodiments, the coupling region of the piezoelectric substrate and the microfluidic substrate is located within or proximate the location where the fluid suspension of particles are to be manipulated and/or sorted within the microfluidic substrate. Thus, for instance, the coupling region may be positioned within or at least near a junction between an inlet microfluidic channel, and two or more outlet microfluidic channels, such that acoustic waves transmitted into the microfluidic substrate through the coupling region are at least sufficient to affect liquid streaming within the microfluidic channels, and in some embodiments such that sorting of droplets or other species is able to occur. In one set of embodiments, there may be three, four, five, or more outlet microfluidic channels, and in some embodiments the sorting of droplets or other species into the two or more outlet microfluidic channels may be controlled by controlling the surface acoustic waves, e.g., by applying suitable voltages to the piezoelectric substrate.

Preferably, the surface acoustic wave has an average frequency of between 100 MHz and 1000 MHz. More preferably, the frequency is 386 MHz. The frequency may vary during use of the microfluidic device. In particular, the spacing between adjacent electrode fingers can gradually change to have a wider working frequency range.

Preferably, the substrate comprises a plurality of outlet channels.

By "channel", it is meant to refer to any feature on or in (or defined) in the substrate that at least partially directs flow of the fluid. The channel can have any cross-sectional shape (circular, oval, triangular, irregular, square or rectangular, or the like) and can be covered or uncovered. In embodiments where it is completely covered, at least one portion of the channel can have a cross-section that is completely enclosed, or the entire channel may be completely enclosed along its entire length with the exception of its inlet(s) and/or outlet(s). A channel may also have an aspect ratio (length to average cross sectional dimension) of at least 2: 1, more typically at least 3: 1, 5: 1, 10: 1, 15: 1, 20: 1, or more. An open channel generally will include characteristics that facilitate control over fluid transport, e.g., structural characteristics (an elongated indentation) and/or physical or chemical characteristics (hydrophobicity vs. hydrophilicity) or other characteristics that can exert a

force (e.g., a containing force) on a fluid. The fluid within the channel may partially or completely fill the channel. In some cases where an open channel is used, the fluid may be held within the channel, for example, using surface tension (i.e., a concave or convex meniscus).

The channel may be of any size, for example, having a largest dimension perpendicular to fluid flow of less than about 5 mm or 2 mm, or less than about 1 mm, or less than about 500 microns, less than about 200 microns, less than about 100 microns, less than about 60 microns, less than about 50 microns, less than about 40 microns, less than about 30 microns, less than about 25 microns, less than about 10 microns, less than about 3 microns, less than about 1 micron, less than about 300 nm, less than about 100 nm, less than about 30 nm, or less than about 10 nm. In a preferred embodiment, the width of the microfluidic channel is about between 10 μm and 1000 μm, and the height of the channel is about 1 μm to 100 μm. In some cases the dimensions of the channel may be chosen such that fluid is able to freely flow through the article or substrate. The dimensions of the channel may also be chosen, for example, to allow a certain volumetric or linear flowrate of fluid in the channel. Of course, the number of channels and the shape of the channels can be varied by any method known to those of ordinary skill in the art. In some cases, more than one channel or capillary may be used. For example, two or more channels may be used, where they are positioned inside each other, positioned adjacent to each other, positioned to intersect with each other, etc.

The wall of the microfluidic channel wall may be of any thickness. In order to minimize the sound attenuation, the wall should be as thin as possible. The wall may be be as thin as 5 μm to 100 μm. This wall thickness refers to the thickness of the material that separates the fluid confined in the channel from the air. Ideally, this wall should be as thin as possible to minimize the wave attenuation. In reality, this wall thickness is from 5-100 um, considering the wave attenuation, fabrication feasibility, and sealability of the channel.

Preferably, the particle is any particle selected from the group: organic particles, inorganic particles, biological cells, and microorganisms. The cells may either be labelled or not prior to sorting/manipulation.

By "cell", it is meant to refer to its ordinary meaning as used in biology. The cell may be any cell or cell type. For example, the cell may be a bacterium or other single-cell organism, a plant cell, or an animal cell. If the cell is a single-cell organism, then the cell may be, for example, a protozoan, a trypanosome, an amoeba, a yeast cell, algae, etc. If the cell is an animal cell, the cell may be, for example, an invertebrate cell (e.g., a cell from a fruit fly), a fish cell (e.g., a zebrafish cell), an amphibian cell (e.g., a frog cell), a reptile cell, a bird cell, or a mammalian cell such as a primate cell, a bovine cell, a horse cell, a porcine cell, a goat cell, a dog cell, a cat cell, or a cell from a rodent such as a rat or a mouse. If the cell is from a multicellular organism, the cell may be from any part of the organism. For instance, if the cell is from an animal, the cell may be a cardiac cell, a fibroblast, a keratinocyte, a heptaocyte, a chondracyte, a neural cell, a osteocyte, a muscle cell, a blood cell, an endothelial cell, an immune cell (e.g., a T-cell, a B-cell, a macrophage, a neutrophil, a basophil, a mast cell, an eosinophil), a stem cell, etc. In some cases, the cell may be a genetically engineered cell. In certain embodiments, the cell may be a Chinese hamster ovarian ("CHO") cell or a 3T3 cell.

In a second aspect of the present invention, there is provided a method for manipulating a particle in a fluid suspension, the method comprising: (a) introducing the fluid suspension along a microfluidic channel; and (b) manipulating the particle using an acoustic source to generate a travelling surface acoustic wave transverse the flow of the fluid suspension in the channel, wherein the acoustic source is an interdigital transducer (IDT), the IDT comprises a plurality of concentric circular arcs having a tapered end directed at the channel, and the tapered end has an aperture of between 4 μm and 150 μm.

In an embodiment, the aperture is 56 μm.

Preferably, the microfluidic channel is defined in a substrate. The substrate may be a piezoelectric substrate, and the piezoelectric substrate may be lithium niobate.

Preferably, the surface acoustic wave has an average frequency of between 100 MHz to 1000 MHz.

Preferably, the particles comprises any particles selected from the group: organic particles, inorganic particles, biological cells, and microorganisms.

In a third aspect of the present invention, there is provided an interdigital transducer comprising a plurality of concentric circular arcs having a tapered end, wherein the tapered end has an aperture of between 4 μm and 150 μm.

In order that the present invention may be fully understood and readily put into practical effect, there shall now be described by way of non-limitative examples only preferred embodiments of the present invention, the description being with reference to the accompanying illustrative figures.

In the Figures:

Figure 1: Schematic illustration of the device design. (a) Sheath flow is used to hydrodynamically focus the sample prior to the focused interdigital transducers (FIDTs), which generate the focused surface acoustic wave (SAW) that translates selected particles. (b) Highly focused SAW is used to sort at the single-particle level, where the width of the focused SAW is on the order of 10's of μm.

Figure 2: Focused SAW and device design. (a) The SAW beam is concentrated using a FIDT structure that results in maximum surface displacements in a defined region ~300 μm from the patterned FIDT features. (b) The fluid channel is bonded directly to the FIDT structure, where the maximum-displacement region is centred on the channel location. (c) SAW displacement velocity, as measured in the maximum displacement region, follows a power law as a function of the input power, here measured in watts. The highly focused nature of the SAW results in large and localized substrate velocities for a given applied power. All scale bars represent 100 μm.

Figure 3: Demonstration of particle sorting using pulsed SAW. (a,b) Individual 3 μm particles in a 1 μl/min flow, bounded by a focusing 8 μl/min sheath flow, are displaced using a 300 μs,

30 mW pulsed focused SAW at 5 ms intervals, which couples into the fluid within an ~25 μm wide beam. (c,d) While the geometry in (a,b) is optimized for sorting using minimal powers and displacements, particles can be translated substantial distances over several wavelengths using travelling SAW. Flow rates of 0.5 and 3 μl/min are used for the particle, buffer flow inlets with SAW pulsed at 100 mW for 1 ms at 10 ms intervals. (a,c) show individual images captured at 10,000 and 5,000 frames per second, respectively, whereas (b,d) show the overlay of 200 frames to demonstrate overall particle trajectories. Images taken from videos. All scale bars represent 50 μm.

Figure 4: Particle velocities in the sorting region. (a) The measured velocity magnitudes of tracked particles not subject to SAW roughly double in the flow constriction region, where the channel width is halved from 80 μm. Inset shows the velocity distributions just prior to (red), in the middle of (green) and after (blue) the flow constriction region, where the variation in velocity magnitudes is a result of the non-uniform (parabolic) flow velocity profile in the *z*-direction. (b) Velocity magnitudes of tracked particles sorted by the focused SAW. Particles are translated laterally within the beam of the tightly focused SAW, approximately 25 μm wide, where particles are translated at approximately 0.15 m/s using 300 μs, 30 mW pulses.

Figure 5: Particle translation behaviour. (a) The percentage of particles translated using a continuously applied SAW is a function of the applied power, here with a total combined flow rate of 6 μl/min. Error bars represent one S.D. from 5 measurements of equal numbers of randomly selected particles (min. 20 selected). Scale bars are 20 μm. (b) The number of particles ejected per pulse is a function of the pulse length for a given particle concentration, here 0.7% v/v, with the average number per pulse defined by Eq. 2. (c) The 1 μm particles are shown travelling through the unsorted outlet meander. Scale bar 400 μm. (d) High frequency affords the sorting of particle sizes not otherwise accessible using travelling waves as per Eq. 1, here demonstrating the selective translation and sorting of 2 μm particles (blue) from 1 μm (green) particles at 4 μl/min with continuously applied 26 mW SAW. Scale bar is 50 μm.

Figure 6: Acoustic streaming in a confined chamber. In these images, 1 μm particles in a static flow condition are subject to a time-averaged flow induced by a body force in the direction of acoustic propagation in two different channel heights (30 μm and 20 μm). Increased acoustic forcing relative to acoustic-induced flow results in greater particle capture in trapping locations on either side of the acoustic beam, where particles are subject to the acoustic gradient force for longer periods in the channel with the smaller height. Scale bar is 100 μm.

Figure 7: Acoustic streaming in flow conditions. As the acoustic intensity is increased in a continuous flow (0.5 μl/min in a 160 μm channel) at (a) 10 mW, (b) 15 mW, (c) 25 mW, (d) 40 mW, in a solution containing 1 μm (green) and 2 μm (orange) particles, the influence of acoustic streaming induced flow relative to particle forces becoming apparent. Here the dominant particle force changes from direct acoustic radiation force (seen shifting the 2 μm particles in (a)) to fluid drag (in (d)), where particles are bound in streamlines according to the superposition of the incident and acoustic streaming flow fields. Scale bar is 100 μm.

In this work we use highly focused travelling SAW to obviate sorting region width limitations in order to generate large and rapid displacements for robust sorting. This localized SAW is produced using a 10 μm wavelength focused IDT (FIDT) structure that generates a beam ~25 μm wide at the narrowest point, sufficient to sort discrete particles using sub-ms SAW pulses without pre-ordering. The device concept is demonstrated in Fig. 1, where Fig. 1b demonstrates the single-particle level sorting that is achievable with a tightly focused translating force field. Furthermore, because of the high-frequency nature of the device generating these highly focused fields, particles as small as 2 μm can be translated and separated. This system has potential use in a wide variety of applications, including the on-demand dilution of particle/cell mixtures and selective cell sorting, as an alternative to the deflection of airborne droplets in FACS.

1. System principles and design

The mechanisms underlying the translation of particles subject to a travelling acoustic wave have been the subject of substantial theoretical work, where several expressions have been

analytically been derived to estimate the directional force they experience.[59-61] In the limiting case where a particle is significantly smaller than the wavelength in the fluid, but not so small that viscous interfacial effects become important, such that the particle radius $R \gg \delta$, where δ is the viscous penetration depth given by $\delta = (2\rho\mu/\omega)^{½}$ and ρ, μ are the fluid density and viscosity and $\omega = 2\pi f$, the formulation from King is sufficient to describe particle motion. The travelling wave force is given by[60,62]

$$F = 64\rho\left(\frac{\omega}{c}\right)^4 R^6 v_0^2 \frac{1+\frac{2}{9}\left(1-(\rho/\rho_p)^2\right)}{\left(2+\rho/\rho_p\right)^2} \quad (1)$$

where c is the speed of sound in the fluid, ρ_p is the particle density and v_0 is the displacement velocity in the fluid, with $v_0 \sim v_{SAW}$. Principally, larger frequencies result in substantially larger forces due to the $F \sim \omega^4$ scaling. The 386 MHz frequency employed here, where the resonant SAW frequency is given by $f = c\lambda$, is thus capable of generating large forces efficiently, even on small particles, to counteract the fact that standing waves typically generate larger force magnitudes for a given fluid displacement velocity.[59,62] The direction of this force, however, has a vertical as well as horizontal component, with the acoustic wave propagating at the Rayleigh angle, where $\vartheta_R = \sin^{-1}(c_l/c_s)$ is this quantity with respect to the orthogonal. In the system described here, with c_s = 3964 m/s and c_l = 1484 m/s, $\vartheta_R \approx 22°$. While the majority of the energy is thus directed vertically, rather than in the horizontal direction, in practice this vertical displacement also serves to aid the horizontal displacement when the force is applied laterally to a continuous flow, whereby the vertical component of the force pushes particles into the slower moving flow near the channel boundaries and thus allowing them to be exposed to the field and thus translated horizontally over a longer period of time.

In Fig. 2 we show the most highly focused SAW transducer yet demonstrated for microfluidic applications, resulting in efficiently generated substrate displacements concentrated in a target area. In the limiting case where the displacement is much less than the wavelength, $\xi \ll \lambda$, the relationship between substrate velocity and applied power follows a power law, given by $v_{SAW} = \beta P^{1/2}$, where β is a material specific coefficient as a function of wavelength and device aperture, and P is the applied power.[63] Due to the small

beam width, the effective β value fit to the substrate velocity measured using laser doppler vibrometry (UHF-120, Polytec, Germany) (see Fig. 2c) is approximately 4x higher than a comparable 30 μm transducer design that produces a ~100 μm wide focused beam.[31]

2. Methods

The sorting system is comprised of a series of FIDTs patterned on a piezoelectric 128° Y-cut lithium niobate (LN, $LiNbO_3$) substrate. The FIDTs are comprised of a 200 nm thick conductive Al layer on top of 7 nm thick Cr layer, which serves as an adhesion layer between the LN and Al. IDTs are patterned in concentric circle segments with a geometric focal point 160 μm from the IDT finger-pair, comprising 36 finger-pairs in total with an aperture of 56 μm at the proximal end up to 210 μm at the distal end, subtending an angle of 26°. The physically realized focusing region does not occur at the geometric one, however, with the maximum displacement and highest degree of focusing occurring ~300 μm from the last finger-pair, as noted in Fig. 2a. Due to the nature of wave propagation in an anisotropic material such as LN, the focusing point is effectively stretched in the crystallographic *x*-direction, the orientation with a minimum power flow angle and maximum propagation velocity,[64] where this effective displacement of the focal point from the geometric one is consistent with previous observations.[65] The entire device is coated with 300 nm SiO_2 using plasma enhanced chemical vapour deposition to prevent corrosion and improve bonding. Fig. 2b shows the completed device, composed of a microfluidic channel bonded directly to the assembled SAW device using plasma activation (PDC 32G, Harrick Plasma, USA).

The device is actuated using A/C pulses generated by a signal generator (APSIN3000HC, Anapico, Switzerland) and amplified by a power amplifier (Model 1100, Empower, USA). Imaging is performed using a high-speed camera (Fastcam Mini UX100, Photron Limited, Japan) on an inverted microscope. Elimination of birefringence effect that would otherwise result in a double-image in an optically anisotropic material is performed by mounting the completed device on a separate piece of double-sided LN in the opposite crystallographic orientation to that of the device. Particle trajectories were recorded using DMV software, a MATLAB-based software package that offers a suite of detection algorithms and parameters to track droplets and particles.[66]

Though PDMS has minimal effect on the resonant frequency of the device,[55] it strongly attenuates SAW displacement at the substrate interface, where the attenuation length is inversely proportional to the wavelength,[67] and is thus an important consideration for the high frequencies used here. The PDMS-attenuating region is minimized by enclosing the SAW transducer in an air-filled chamber, with only a 20 μm wide PDMS wall separating this region from the liquid filled channel. The flow constriction serves to further minimize the distance the SAW is subjected to any attenuating interface prior to particle interaction and also to reduce the chance that multiple particles will be translated simultaneously by stretching the flow locally. As noted in Fig. 1a, the device has only two independent inlets and outlets, where both sheath and $outlet_2$ channel flows are hydrostatically balanced though the use of equidistant channel lengths upstream and downstream. The channel, fabricated using PDMS (10:1 elastomer/curing agent ratio) soft lithography on an SU-8 mould, has a height of 20 μm.

To perform sorting, particle-free buffer flow and a particle solution containing 3 μm particles are injected in the sheath and sample inlets, respectively, using separate syringe pumps (NE-1000, New Era Pump Systems, Inc., NY, USA and Legato 111, KD Scientific, MA, USA). The sorting designs used have two independent outlets, $outlet_1$ and $outlet_2$. When a particle is in the path of the focused SAW at sufficient amplitude, it is translated from the sample stream into fluid flow that exits via $outlet_2$, as opposed to unsorted particles that exit via $outlet_1$.

3. Results and Discussion

Particle sorting can be accomplished over a minimal sorting width using focused SAW, where the beam width is minimized by using a small wavelength (10 μm), small aperture (56 μm at minimum) FIDT structure that results in a highly localized maximum displacement region. Further, displacements are maximized in utilizing travelling SAW, as opposed to standing SAW; at this wavelength a standing wave would yield a theoretical maximum displacement of 2.5 μm, as opposed to much larger translations possible with a travelling wave for a given wavelength, though the force magnitude is maximized in the near field due to the attenuation at the substrate/fluid interface with an attenuation length (at which the

substrate velocity decays to 1/e) of approximately 12λ.[68] We demonstrate both of these characteristics, where individual 3 μm particles are displaced with the application of a SAW. Pulses with periods $t_p \lesssim 1$ ms are utilized, similar to the pulse lengths in other work,[36,37,46,47] as minimizing the SAW exposure time in a continuous flow reduces the chance of unintended sorting events (false-positives). Fig. 3 shows that the highly focused beam, with an aperture of ~25 μm, is compatible with different device geometries, here using a flow focusing geometry (Fig. 3a,b) and a H-filter arrangement (Fig. 3c,d), where Figs. 3b,d show the trajectories of sorted and unsorted particles as a continuous particle stream passes through a pulsed SAW.

The fact that substantial displacements, ~50 μm in Fig. 3d, can be generated using short SAW pulses at low powers ($\lesssim$ 100 mW) further reflects the advantages of a focusing aperture and a high-frequency (386 MHz) SAW that generates larger travelling wave forces as per Eq. 1, and 3 μm particles can be deterministically displaced more rapidly and at lower power levels than demonstrated elsewhere.[42] Partially displaced particles that do not exit via outlet2 are those subject to a sub-threshold acoustic force at the edge of the focused beam, outside the sorting region.

The power required is minimized by limiting the lateral displacement needed for sorting, hence the geometry in Fig. 3a requires lower powers for sorting at equivalent flow rates due to the 40 μm wide flow restriction region in the centre of the channel, half the 80 μm width immediately upstream and downstream. The effect of this flow restriction is reflected in the velocities of particles tracked through this region. Fig. 4a shows the velocity magnitudes of unsorted particles in the focused sorting region (those exiting through outlet1) where the average velocity magnitude doubles. Though flow focusing aligns the particles in the y-direction, a parabolic flow profile (resulting from the no-slip boundary condition at the fluid/channel interface) yields a range of particle velocities in the z-direction. The dotted lines in this figure show the particle velocity magnitudes of tracked particles, where the inset in Fig. 4a shows a histogram of these velocities. If the acoustic force acted solely in the direction of SAW propagation, this range of velocities would necessitate substantially higher powers to deterministically translate all particles, especially the fastest moving ones. As the θ_R that results from a LN/H_2O interface is directed principally in the direction normal to the

surface, however, the velocity disparities are quickly eliminated, where particles are first pushed against the channel roof before being translated laterally. This is demonstrated in Fig. 4b, where despite a range of particle velocities prior to sorting, the maximum velocity magnitudes are equalized around approximately 15 cm/s with an applied power of 30 mW. Importantly, this feature of acoustic coupling also results in power-efficient translation, where the slower flow at the channel interface results in longer exposure times to the acoustic beam, resulting in equivalent lateral translation velocities for same-sized particles. The maximum translation velocities, effectively comprised of sole translation in the lateral direction, occurs within the ~25 μm wide sorting region. At the cessation of the SAW pulse, particles continue with the flow in the sorted outlet at a relatively uniform and low velocity, reflecting the low flow velocity at the fluid/channel interface.

The percentage of sorted particles can be directly attributed to both the power and length of the applied pulses; while characterizing the effects of these parameters is important for understanding the powers and pulse times required for sorting, modifying these parameters can also be used to perform selective fractionation. Fig. 5a shows the effect of increasing applied power on the percentage of particles sorted, as measured at the outlets (particle solution contains 1 μm tracer particles that are not sorted, whose path through the system is shown in Fig. 5c), where the quick transition from no sorting to near-complete sorting occurs due to the aforementioned velocity equalization with acoustic forcing at ϑ_R. Sorting percentage is measured by the number of particles counted (min. 102 total) in $outlet_1$ and $outlet_2$ at different power levels (0-10 mW) from an input of 3 μm particles subject to a constantly applied SAW. The quantity of particles dispensed can be more deterministically defined, however, by changing the pulse length. Fig. 5b demonstrates that the number of particles sorted per SAW pulse is a linear function of the pulse length, where any number of particles in the sorting volume *Vs* when a SAW pulse is generated are translated; additional particles arriving in this region in the continuous flow while a SAW is generated will also be sorted. The slope of this line is a function of the particle concentration *C*, here 0.07% vol/vol, with the average number of dispensed particles given by

$$N = CV_s\left(1 + \frac{t_p v_f}{w}\right) \quad (2)$$

where t_p is the pulse time, w is the sorting region width and v_f is the average flow velocity in the sorting region, provided the pulse lengths are at least as long as the time it takes for a particle to translate a sufficient distance to be sorted ($t_p \gtrsim$ O(100 μs)).

An important consequence of using high frequencies is that substantially larger forces can be generated for equivalently sized particles subject to a travelling wave, as noted by the scaling in Eq. 1. This is demonstrated in Fig. 5d, where fluorescent 2 μm particles are separated from a solution with 1 μm particles in a continuous flow. This is the smallest size yet shown to be displaced using a travelling SAW, where recent work demonstrated the translation of particles as small as 3.2 μm using a 200 MHz field.[42] As SAW is readily capable of generating fields up to the GHz scale, this points the way to using travelling wave forces to potentially manipulate even smaller objects.

Not yet addressed is the potential for acoustic streaming to affect the flow profile in the vicinity of the focused acoustic beam. Acoustic streaming is a phenomena caused by the attenuation of the acoustic displacement amplitude as it passes through a dispersive media, including water, resulting in momentum transfer in the direction of acoustic propagation[68]. This phenomenon can be used to drive fluid flow for pumping or mixing[65] or particle concentration, as demonstrated in Figure 6. Here 1 μm particles are concentrated in the closed fluid streamlines in the vicinity (on either side) of the acoustic beam. This phenomenon can be understood in the context of the acoustic radiation force, where the acoustic force gradient prevents particles from passing through the region of maximum beam intensity[69], resulting in these particles being shifted into tighter streamlines with every pass through the focusing region. The magnitude of the acoustic force relative to the flow induced by acoustic streaming can also be altered, with viscous-shear induced drag (caused by the no-slip channel boundary conditions at the channel interfaces) resulting in an inverse relationship between flow velocity and channel height. This greater proportion of acoustic radiation force over fluid drag results in more complete particle capture in the case of smaller channel heights.

Figure 7 shows the effect of increasing acoustic streaming on the trajectory of particles through an acoustic beam. Fig. 7a demonstrates the flow regime necessary for particle sorting, where the acoustic force results in particle displacement (here displacing 2 μm particles in combined 2+1 μm particle solution) without substantive disturbance to the flow profile. At higher applied powers in the same flow, acoustic streaming contributes more to the local velocity profile, leading to a transitional regime (Fig. 7d) where particles are displaced both according to the direct acoustic force, but also with the fluid streamlines in the vicinity of the acoustic beam. This opens the possibility for not only particle sorting, but also particle capture and concentration in a continuous flow – changing the ratio between the flow velocities induced by acoustic streaming and those from the continuous input flow shifts the regime from one where the acoustic particle force is dominant to one where fluid drag determines particle behaviour. Acoustic streaming, however, does not substantively affect the flow profile in high throughput sorting, whose flow velocities exceed those induced by the travelling wave acoustic forces necessary to induce particle displacement (of >2 μm particles).

4. Conclusions

SAW has rightly been applied to a wide number of microfluidic applications, where their ability to generate substantial and biocompatible forces on particles, cells and droplets is an essential feature. Further, because these forces can be generated and applied locally, it is straightforward to integrate compartmentalized SAW systems with other microfluidic processes. Microfluidic high-speed sorting is one such application where SAW is ideally suited, where sufficient forces can be generated on cells using sub-ms pulses to translate them. Minimizing the width of the sorting region is essential to deterministic translation, where minimum-width standing wave transducers and limited PDMS posts have been previously demonstrated. For robust and reliable sorting in a variety of sample concentrations the sorting region should be comparable to the size of cells being processed.

In this work we have demonstrated the use of highly localized acoustic fields generated by focused SAW for single-particle level displacement, where deterministic sorting is made

possible using a focused beam with a width of only 25 μm. As the minimum beam width is by necessity on the scale of the wavelength or larger, this is realized using a high-frequency, 386 MHz, 10 μm wavelength set of FIDTs. Using pulses on the scale of 100's of μs, sorting rates between 1-10 kHz can theoretically be achieved. Further, because objects with diameters down to 2 μm can be translated on-demand using this frequency, varying pulse lengths can also be used to create time-varying particle concentrations. While this demonstration does not include a sensing apparatus, the integration of such an optical detection system is relatively straightforward in optically transparent materials such as LN/PDMS, or where detection based on impedance is possible through the integration of suitable electrodes.[70] With such integration, highly focused SAW is ideally suited to deterministic sorting and microfluidic manipulation, potentially with specimens down to the scale of bacteria. Futhermore, proper control of the applied power on the FIDTs and the flow rate of the continuous input flow can also incorporate the acoustic streaming in the vicinity of the focused acoustic beam for particle capture and concentration in a continuous flow.

Whilst there has been described in the foregoing description preferred embodiments of the present invention, it will be understood by those skilled in the technology concerned that many variations or modifications in details of design or construction may be made without departing from the present invention.

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Claims

1. A microfluidic device for manipulating a particle in a fluid suspension, the device comprising:

(a) a substrate;

(b) a channel defined in the substrate, the channel having an inlet for receiving the fluid suspension and an outlet for discharging the fluid suspension; and

(c) an acoustic source configured to deliver a travelling surface acoustic wave transverse the flow of the fluid suspension in the channel,

wherein the acoustic source is an interdigital transducer (IDT), the IDT comprises a plurality of concentric circular arcs having a tapered end directed at the channel, and the tapered end has an aperture of between 4 μm and 150 μm.

2. The device according to claim 1, wherein the aperture is 56 μm.

3. The device according to any one of claims 1 or 2, wherein the substrate is a piezoelectric substrate.

4. The device according to claim 3, wherein the piezoelectric substrate is any piezoelectric material selected from the group comprising: lithium niobate, lithium tantalite, and lanthanum gallium silicate.

5. The device according to any one of the preceding claims, wherein the surface acoustic wave has an average frequency of between 100 MHz and 1000 MHz.

6. The device according to any one of the preceding claims, wherein the substrate comprises a plurality of outlet channels.

7. The device according to any one of the preceding claims, wherein the particles comprises any particles selected from the group: organic particles, inorganic particles, biological cells, and microorganisms.

8. A method for manipulating a particle in a fluid suspension, the method comprising:

(a) introducing the fluid suspension along a microfluidic channel; and

(b) manipulating the particle using an acoustic source to generate a highly focsed travelling surface acoustic wave transverse the flow of the fluid suspension in the channel,

wherein the acoustic source is an interdigital transducer (IDT), the IDT comprises a plurality of concentric circular arcs having a tapered end directed at the channel, and the tapered end has an aperture of between 4 μm and 150 μm.

9. The method according to claim 8, wherein the aperture is 56 μm.

10. The method according to any one of claims 8 or 9, wherein the microfluidic channel is defined in a substrate.

11. The method according to claim 10, wherein the substrate is a piezoelectric substrate.

12. The method according to claim 11, wherein the piezoelectric substrate is lithium niobate.

13. The method according to any one of claims 8 to 12, wherein the surface acoustic wave has an average frequency of between 100 MHz and 1000 MHz.

14. The method according to any one of claims 8 to 13, wherein the particles comprises any particles selected from the group: organic particles, inorganic particles, biological cells, and microorganisms.

15. An interdigital transducer comprising a plurality of concentric circular arcs having a tapered end, wherein the tapered end has an aperture of between 4 μm and 150 μm.