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Acoustofluidics 5: Building microfluidic acoustic resonators

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Acoustophoresis is getting more attention as an effective and gentle non-contact method of manipulating cells and particles in microfluidic systems. A key to a successful assembly of an acoustophoresis system is a proper design of the acoustic resonator where aspects of fabrication techniques, material choice, thickness matching of involved components, as well as strategies of actuation, all have to be considered. This tutorial covers some of the basics in designing and building microfluidic acoustic resonators and will hopefully be a comprehensive and advisory document to assist the interested reader in creating a successful acoustophoretic device.

Introduction

The combination of microfluidics and acoustic standing wave technology has become a viable route to develop integrated systems for non-contact and in-chip manipulation of cells and particles. Acoustic standing wave technology offers means to move and spatially localise cells and particles by utilising acoustic standing wave forces in an acoustic resonator *i.e.* acoustophoresis. As acoustophoresis is performed in a continuous flow based system commonly a mode of separation is sought where particles having different acoustophysical properties can be differentiated. Acoustophoresis can also be utilised to induce retention against flow or aggregation of particles in defined locations, which commonly is performed in so-called acoustic traps (or acoustic tweezers). Acoustic trapping allows for *e.g.* detailed investigations of free floating cell aggregates over an extended period of time. A fundamental requirement to accomplish these modes of operation is that the microfluidics system is designed with well-defined acoustic resonators.

A classical way of designing an acoustic resonator for cell and particle handling comprises a compartment where one wall of the resonator incorporates a piezoceramic transducer glued to a coupling layer of glass or metal and the opposing wall serves as a passive reflector,[1] commonly referred to as a layered resonator. Optionally the opposing wall can be supplied with a second transducer operating at the same frequency as the first transducer,[2] Fig. 1. These resonators were typically operated in a multi-node resonance mode and of centimetre dimensions or larger.[3,4]

As half wavelength resonators started to appear, as described by Mandralis *et al.*[5] in 1990 and experimentally presented[6] in 1993, dimensions were inherently reduced to a couple of hundreds of micrometres, bringing the system fluidics into a mode of low Reynolds numbers and hence a laminar flow domain.[2,7,8] The dimensional reduction also follows with the scaling laws of the primary axial radiation force,[9,10] which in a simplified version described as a one-dimensional resonance mode is expressed according to eqn (1). The acoustic force is proportional to the employed frequency and hence by reducing the resonator dimension to a half wavelength resonance condition, the frequency will have to be increased. An increased frequency yields a higher magnitude of the manipulating acoustic force, which thus increases to a level where strong forces enable manipulation of particles/cells in continuous flow based systems. Consequently, the combination

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Foreword

The fifth paper of 23 in the tutorial series on Acoustofluidics in *Lab on a Chip* presents guidelines for building microfluidic acoustic resonators. Although there are many types of acoustic devices designed for different purposes, they have many parameters in common. The choice of material, design configurations and various strategies of actuation are all important to consider in order to conceive an acoustofluidic device. Further, different types of acoustofluidic devices such as layered resonators, transversal resonators, surface acoustic wave devices and acoustic traps are discussed.
Andreas Lenshof – coordinator of the Acoustofluidics series

of microfluidics systems and acoustic standing wave manipulation lends itself well to microsystem integration.

$$F_{\mathrm{Ax}} = 4\pi R^3 Ek\sin(2kx)\Phi \quad (1)$$

where

$$\Phi = \frac{\rho_\mathrm{p} + (2/3)(\rho_\mathrm{p} - \rho_0)}{2\rho_\mathrm{p} + \rho_0} - \frac{1}{3}\frac{\rho_0 c_0^2}{\rho_\mathrm{p} c_\mathrm{p}^2} \quad (2)$$

where F_{Ax} = primary axial radiation force, E = acoustic energy density, R = particle radius, x = particle position in the wave propagation direction, ρ_p and ρ_0 = density of particle and fluid respectively, c_p and c_0 = speed of sound in the particle material and fluid respectively, $k = 2\pi f/c_0$, Φ = acoustic contrast factor, f = frequency.

Early work by Yasuda *et al.* in 1992 described the use of half wavelength resonators in the MHz regime.[11] The patent outlined precise focusing of cells in the standing wave node in the flow channel and further downstream acoustically enriched fractions of particles were selectively collected in the cell enriched flow segment, Fig. 2.

The combination of acoustic control of particle location in a fluid and the simultaneous transition to a laminar flow domain in half wavelength resonators has opened up acoustic particle handling to become a powerful means to manipulate particles and cells in microfluidic systems.[12,13]

A key feature of this is the ability to allow solely the geometrical dimensions and material properties to govern the fundamental acoustic forces that allow cell or particle manipulation. An important consequence is that the acoustic

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Professor Thomas Laurell holds a position as Professor in Medical and Chemical Microsensors and has since 1995 built his research activities around microtechnologies in biomedicine (http://www.elmat.lth.se/forskning/nanobiotechnology_and_labonachip). Laurell recently started a new applied nanoproteomics laboratory at the Biomedical Centre in Lund, integrating microfluidics and nanobiotechnology developments with medical research. This research is focused on new microchip technologies in the area of biomedicine, biochemistry, nanobiotechnology with a focus on disease biomarkers, diagnostic microsystems and miniaturised sample processing. Laurell also leads the clinically oriented research environment CellCARE, (www.cellcare.lth.se), which targets chip based cell separation utilising ultrasonic standing wave technology (acoustophoresis) as the fundamental mode of separation.

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Mikael Evander received his PhD degree in Electrical Measurements at Lund University in 2008. He was a postdoctoral fellow at the Department of Electrical Engineering at Stanford University between 2009 and 2011 and is currently employed as a researcher at the department of Measurement Technology and Industrial Electrical Engineering at Lund University. His research is focused on technologies for cell monitoring and manipulation in lab-on-a-chip systems, e.g. *cell and particle trapping using acoustic radiation forces and impedance spectroscopy of single cells.*

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Johan Nilsson holds an MSc in Electrical Engineering and a PhD in Electrical Measurements, both from Lund University, Sweden. He is an Associate Professor and since 2000 has been the Head of the Department of Measurement Technology and Industrial Electrical Engineering at Lund University. His teaching activities include courses in Electrical Measurements, EMC and Microfluidics. His main research topics are microfluidics and microsystems with a focus on droplet based processes and acoustic trapping for bio-analysis.

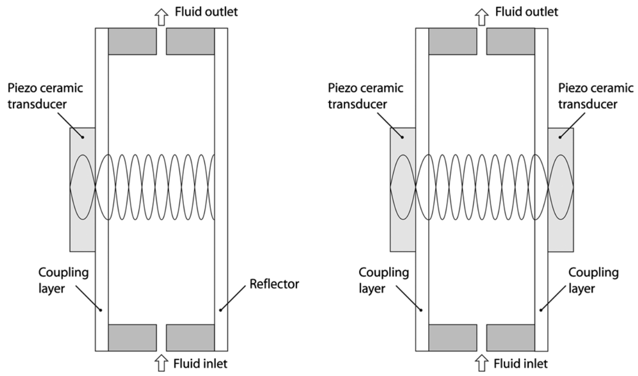

Fig. 1 Classic configurations of a layered resonator with either a single transducer and a reflector layer or two opposing transducers.

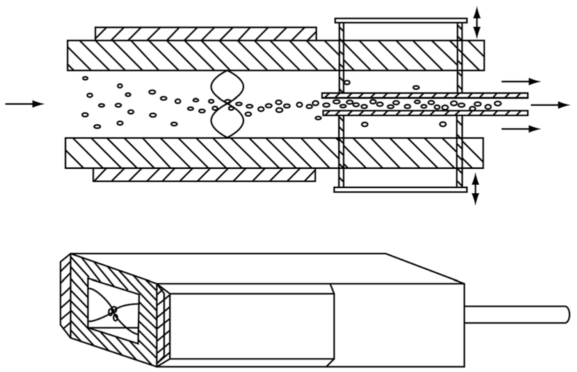

Fig. 2 Half wavelength acoustic resonator for particle and cell focusing as disclosed by Yasuda *et al.*[11]

forces acting on the particles/cells are to a large extent independent of properties such as ionic strength, pH or surface charge, hence making acoustophoresis a generic tool to manipulate biological suspensions, be that blood, plasma, urine, fermentation broths, milk, cell cultures *etc.* Thus the mere design and composition of the resonator in terms of geometrical parameters and material choice becomes a key engineering challenge to accomplish efficient acoustic resonators for particle and cell manipulation. This tutorial will review the literature and outline basic considerations that have to be made when building acoustic resonators for continuous flow-based acoustophoresis or resonators for acoustic cell/particle trapping.

Choice of material

The choice of material is very important when designing an acoustic resonator. However, the type of resonator system also influences the material possible to use. There are three main types of acoustophoretic systems, the layered resonator, the transversal resonator and the surface acoustic wave (SAW) resonator.

The layered resonator is a quite complex resonator as the different layers require precise control of thicknesses in order to achieve a resonator with high Q-value, Fig. 3. However, as it is the system Q-value that is important, there is actually room for using materials that are often not considered as good resonator materials, such as polymers that are known for their high acoustic attenuation. As the main reflection occurs between the air backed reflector layer and the transducer, some losses in the supporting layers can be acceptable in order to maximize the energy density in the fluid layer. The attenuation in the polymers can also be an advantage as it results in resonators with larger bandwidths.

The transversal separator, on the other hand, relies on reflections between the channel walls and requires materials of high characteristic acoustic impedance, such as glass, silicon or metals. The high Q-value materials make this resonator type less sensitive to different thicknesses and matched layers as it is the entire bulk that resonates as one body, even though the bonding of the lid to the bulk structure is important to minimize attenuation of the resonance.

The resonance in SAW devices is created differently as they rely on waves propagating into a fluidic compartment *via* a wave guiding substrate. In order not to create interfering resonances, the material enclosing the fluid should be of similar characteristic acoustic impedance as the fluid, making polymers suitable.

A "good" resonator material is thus very device specific. There are some tools that can be useful when designing the acoustic resonator, such as the acoustic impedance. The characteristic acoustic impedance (Z) is comprised of the density (ρ) and speed of sound (c) of the material according to eqn (3).

$$Z = \rho c \tag{3}$$

The characteristic acoustic impedance is useful for calculating the reflection and

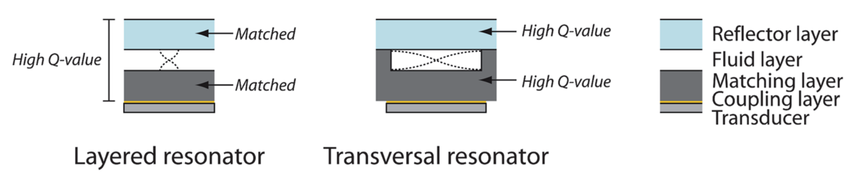

Fig. 3 The choice of material depends on what type of resonator is to be designed. The layered resonator requires carefully matched reflection and matching layers with regards to the wavelength in order to achieve a system with high Q-value. Although, as it is the system Q-value that is important here, it is possible to use materials which themselves are not acoustically optimal, such as polymers, and some losses could be acceptable as long as the system is well matched. Transversal resonators on the other hand rely more on materials with high characteristic acoustic impedance and are less sensitive to matched layers as the whole system resonates as one body. They are thus easier to design, but are limited to the choice of materials which can be utilised.

transmission coefficient.[14] For normal incidence, the pressure reflection coefficient is:

$$R_p = \frac{Z_2 - Z_1}{Z_1 + Z_2} \quad (4)$$

where Z_1 is the characteristic acoustic impedance of the first medium and Z_2 of the second. The transmission coefficient of a wave of normal incidence is subsequently

$$T_p = 1 - R_p \quad (5)$$

To avoid acoustic losses due to reflection, the acoustic impedances of two adjacent layers should be carefully matched so that the acoustic energy density in the fluidic layer is maximised. For instance, when designing the matching layer, the characteristic acoustic impedance of the layer should be lower than that of the transducer but higher than the material comprising the fluidic cavity.

There have been numerous different material combinations in acoustophoretic devices over the recent years. It should be noted that these materials have very different material properties, as can be seen in Table 1. One of the most common material choices is to make the flow channel in silicon.[15,16] The monocrystalline structure of silicon enables precise structures to be fabricated using etching techniques. For example, with the correct mask alignment with regards to the etch planes in wet etching, it is possible to achieve channels with vertical walls, which are good for standing wave acoustics.[15] A borosilicate glass lid to seal the flow channel is preferable as it provides good visual access to the flow channel and the anodic bond process provides a very strong electrochemical bond that makes the two pieces resonate as one body.

When designing transversal resonators it was initially thought that a resonator channel with a homogeneous width, *i.e.* vertical walls, was a prerequisite for standing wave resonances to occur and that an amorphous material such as glass, which gives a semi-circular cross-section if wet etched, was not suitable. Glass itself has very good properties for standing wave acoustofluidics as it has a quite large difference in density compared to aqueous fluids and reasonably high speed of sound, as well as being chemically inert. Evander *et al.* showed that a resonator consisting of a wet etched flow channel in glass, covered with a fusion bonded glass lid, showed similar performance to silicon channels,[17] even though there was the substantial difference in the channel cross-section appearance.

From a material properties point of view, steel is another suitable resonator material. Although not very commonly used, as the microfabrication processes which are used with silicon and glass are not applicable, Hawkes and Coakley reported a layered steel resonator.[18] The resonator was made through wire erosion, although the flow channel itself was defined by a rubber gasket and the height determined by a brass spacer. Steel has high density, good sound propagating properties and also has the advantage of good heat transport capabilities.

Polymers have many desirable properties such as low cost and easy mass fabrication, such as injection moulding, making them suitable for disposable devices to be used for medical and clinical purposes. Unfortunately, polymers are less optimal from an acoustical point of view. A polymer/water interface shows poor acoustic reflective abilities, which is undesirable when designing transversal resonators and the relatively high attenuation of acoustic waves makes it more difficult to create standing waves without heavy losses. However, PMMA/SU-8 devices have proven to be useful in layered resonators where the intention is to have the nodal plane less than a ¼ wavelength from the wall.[19] A way to create standing waves without being limited to the restricted reflection abilities of polymers is to use two opposing transducers. This enabled the use of soft polymers such as PDMS since the resonator is defined by the two actuators.[20] In other resonator configurations it would be difficult to set up a standing wave in a cavity of this material as the acoustic impedance of PDMS is close to water. This property is, however, exploited in surface acoustic wave (SAW) devices where PDMS is used as a channel material on top of a piezoelectric substrate.[21,22]

Table 1 Density, speed of sound and characteristic acoustic impedance for some materials

	Density/ kg m^{-3}	Speed of sound/m s^{-1}	Characteristic acoustic impedance/10^6 kg m^{-2} s
Silicon	2331	8490	19.79
Pyrex	2230	5647	12.59
Steel—stainless 347	7890	5790	45.68
Aluminium	2700	6420	17.33
Titanium	4506	6070	27.35
Polymethyl methacrylate (PMMA)	1150	2590	2.98
Polycarbonate (PC)	1200	2160	2.59
Polystyrene (PS)	1050	1700	1.79
Polydimethylsiloxane (PDMS)	965	1076 (10 : 1)	1.04
		1119 (5 : 1)	1.08
H_2O (25 °C)	997	1497	1.49
PZT transducer	7700	4000	30.8
Air	1	343	0.00034

Design configurations

The first thing to consider when designing the resonator is the size range of the particles to be manipulated. The most common and practical range is about 1 μm to 20 μm in diameter. For sizes below 1 μm, the primary radiation force is quite weak as it scales with the volume of the particle, and at that level other forces such as viscous drag from acoustic streaming will start to dominate over the primary radiation force. At sizes above 20 μm, gravity will start to affect the particles even more and sedimentation may occur, although the rate is highly dependent on the densities of the particles compared to the fluid medium. The force on the particle is also proportional to the acoustic frequency, see eqn (1). Smaller particles that are less affected by the radiation force can still be manipulated by choosing a higher frequency. However, the wavelength decreases with the increasing frequency, meaning that the width of the fluid layer has to decrease accordingly. It must also be decided if the device is to work at the fundamental frequency (*i.e.* a single pressure node) or at a higher harmonic (multiple pressure

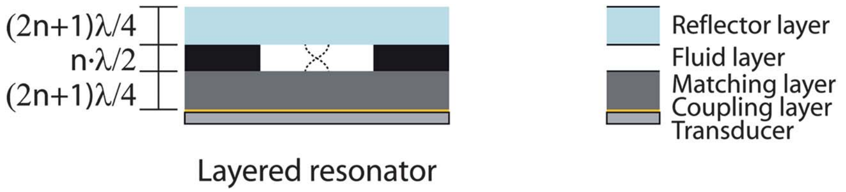

Fig. 4 The structure of a layered resonator.

nodes). Although multiple node resonators can sometimes be beneficial,[23] the most common systems use a half-wavelength resonator. When perfused continuously these devices yield a single particle band located in the center of the fluidic channel. Generally speaking, a frequency range of 1–10 MHz is suitable for the manipulation of particles in the range of 1–20 μm.

Layered resonators

A layered resonator is a structure composed of different layers which all have a very specific role in the resonator system as the sound wave passes through them, building up the resonance. The different layers can be seen in Fig. 4. The transducer generates the sound and is followed by the coupling layer, which is needed in order to get good acoustic transmission into the system. Next follows the matching layer, sometimes called the transmission layer, which forms the bottom of the resonator chamber and thus also acts as a reflector surface of the standing wave. The fluid layer contains the liquid and cells or beads. At the other end of the system is the reflector layer that is responsible for reflecting the incoming wave back into the fluid layer, giving rise to the standing wave.

The thicknesses of the different layers in the layered resonators are of great importance in order to get as powerful resonance as possible. Hawkes *et al.* have shown in simulations that a good half wavelength layered resonator should be constructed as follows: a matching layer of a quarter wavelength, fluid layer of half a wavelength and a reflector layer of a quarter wavelength thickness.[7] The quarter wavelength in the matching layer is chosen to maximize the Q-value of the layered resonator. The quarter wavelength in the reflection layer is chosen because of the phase shift of the wave when it reflects to a less dense medium (air). This configuration results in a pressure minimum in the center of the fluidic channel and a pressure maximum at the channel walls, a configuration used in most cell manipulation applications. However, if other locations of the pressure node are desired, such as in the application by Hill[24] where a pressure node is located at one of the walls in a quarter wavelength configuration, other layer parameters are preferable. As this resonance system is not as stable as the half wavelength systems, simulations are needed to predict the layer thicknesses for the special frequency conditions required.[25] The quarter wavelength fluid cavity can be used to drive cells towards a surface which is modified for capturing specific particles.[26]

Transversal resonators

In contrast to the layered resonator, the transversal resonator is operated such that a standing wave perpendicular to the incident direction of actuation is obtained, Fig. 5. By exciting the resonator structure at a frequency that matches a half wavelength criterion with respect to the channel width, a transversal mode of operation can be accomplished. Historically, the transversal mode of operation has predominately been reported in resonators made of high Q-value materials, such as glass, silicon or metal. Since the entire microfluidic component is actuated in the transversal mode, a general advice is that the resonator in this case is made of materials with low acoustic losses that display a high difference in characteristic acoustic impedance *versus* the fluid. By matching the dimension of the bulk material enhanced operating characteristics can be obtained with good acoustic focusing properties.[10] A clear benefit of the transversal resonator configuration is that the acoustic manipulation is performed in plane with the resonator chip, facilitating visual observation of the focusing event. Also, since the actuation is performed in plane with the chip, integration with other microchip based fluidic functions is easily accomplished.

SAW devices

Surface Acoustic Wave (SAW) devices utilise surface waves to manipulate particles in a channel. The surface waves are generated by one or more interdigitated electrode transducers positioned outside of the channel, normally fabricated in PDMS using soft lithography,[27] Fig. 6. The interdigitated electrode transducers are created by forming metal strips on a piezo-electric substrate, commonly $LiNbO_3$, by metal evaporation and lift-off. The PDMS channel is fabricated by moulding PDMS on a silicon or SU-8 master. The PDMS channel mould is then bonded against the transducer wafer/plate. The basic principle and an example of a device are shown in Fig. 6.

In the most straightforward configurations the inter-electrode distances, λ_{SAW}, are given by the frequency, f, and the surface wave-velocity in the piezo-electric material, v_{SAW}, according to:

$$\lambda_{SAW} = v_{SAW}/f \tag{6}$$

By using opposing transducers, a standing surface acoustic wave (SSAW) is generated in the substrate that couples

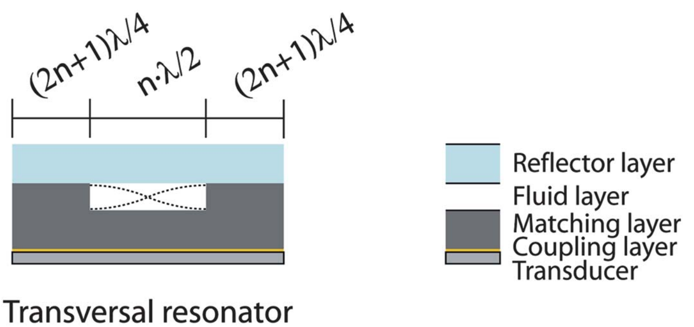

Fig. 5 The structure of a transversal resonator.

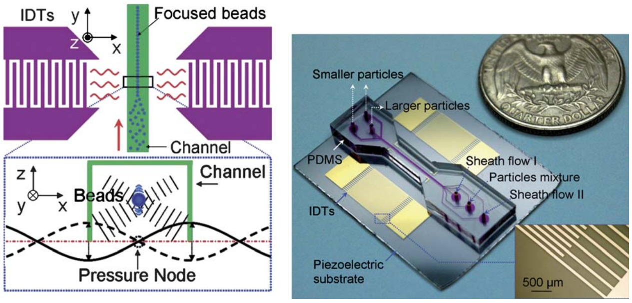

Fig. 6 Left: basic principle for bead focussing using SSAW.[21] Right: example of an SSAW device with a channel fabricated in PDMS.[22] Reproduced with permission of the Royal Society of Chemistry.

into the channel and generates the pressure field. Although the PDMS/water interface does not compose a resonant system, the phase relationship between the two opposing fields entering the channel will determine the position of the SSAW in the channel. This opens up the possibility of controlling the positioning of the particles within the channel by individually controlling the phase of the signals to the transducers. Since the transducers are situated at a distance equal to several wavelengths away from the channel, accurate temperature control may be an issue due to the temperature dependence of the surface wave velocity in the substrate. A more in depth discussion on SAW-devices for microfluidic acoustic particle manipulation is presented in the Acoustofluidics tutorial no. 17.

Flow splitter design

A flow splitter is an essential component in many acoustophoretic applications, *e.g.* for collecting and maintaining the integrity of separated species. Different flow splitter designs fabricated using anisotropic etching of silicon can be seen in Fig. 7. The geometric design of the flow splitter is of great importance for the operation.[28] Generally, as with most microfluidic devices, sharp 90° corners should be avoided as gas bubbles are prone to gather there, disturbing the flow.[15] Also, sharp features, such as ridges or flow splitters, can induce considerable acoustic streaming that may interfere with the laminar flow.[29] However, when working with crystalline materials such as silicon there are certain limitations to which designs are possible, at least if the anisotropic wet etching properties of silicon are to be utilised. The smaller the angle of your flow splitter, the less likely it is that a gas bubble will actually get stuck in your flow channel, which makes the 45° in Fig. 7b preferable over Fig. 7a. The splitters in Fig. 7a and b are etched on the same wafer but along different crystal orientations. The 90° splitter in Fig. 7a has vertical walls in all channels while the 45° splitter in Fig. 7b has slanting walls in the side channels. The splitter in Fig. 7c is etched in ⟨110⟩ silicon which is more challenging than ⟨100⟩ silicon as the etch planes are not symmetrical and thus limit the downstream fluidic network design possibilities.

Acoustic traps

In a resonator designed for an acoustophoresis system, the axial primary radiation force is commonly utilised to translate objects into the pressure node and the fluid flow subsequently transports the particles to *e.g.* a flow-splitter. In a trapping system, however, the goal is to use the acoustic forces to retain particles against a flow at a fixed position in a channel. Apart from the axial component of the primary radiation force, this setup relies heavily on the lateral component of the primary radiation force to hold the particles in place. The acoustic radiation force is based on both the pressure and the velocity gradient in the standing wave and in order to achieve an efficient trap, large, lateral gradients will be required. The force resulting from the lateral velocity gradient for a dense particle with a radius, R, situated in the pressure node of a standing wave with constant amplitude gradient can be expressed as:[30]

$$F_{\mathrm{LAT}} = \pi \rho \omega^2 R^2 u_0 u_{\mathrm{m}} \qquad (7)$$

ρ denotes the fluid density, ω the angular frequency of the ultrasound, u_{o} is the displacement amplitude at the center of the particle and u_{m} is the difference in displacement amplitude at the edge of the particle compared to the center. As can be seen, the lateral component is not as size dependent as the axial component of the radiation force and the greater the difference in amplitude between the center and the edge of the particle, the larger the trapping force. So in order to design a strong lateral trap, a large, lateral gradient is needed.

Another force that is more important in trapping systems than in acoustophoresis systems is the secondary radiation force that is created from the main acoustic field scattering on the objects that are being trapped. The force is strongly dependent on the distance between the particles ($1/d^4$) and therefore mainly acts on the particles when they are already focused into the pressure nodal plane by the primary radiation force and brought into close contact (10–100 µm) by the lateral component of the primary radiation force.[30] The secondary force attracts particles to each other and helps to build and stabilize the trapped cluster.

In order to create a strong lateral gradient, trapping systems are usually

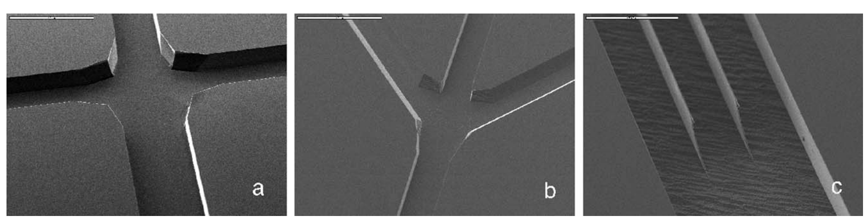

Fig. 7 (a) Silicon flow channel with 90° flow splitters etched on a ⟨100⟩-oriented silicon wafer. (b) Silicon flow channel with 45° flow splitters etched on a ⟨100⟩-oriented silicon wafer. (c) Silicon flow channel with straight flow splitters etched on a ⟨110⟩-oriented silicon wafer. From Laurell *et al.*[28] Reproduced with permission of the Royal Society of Chemistry.

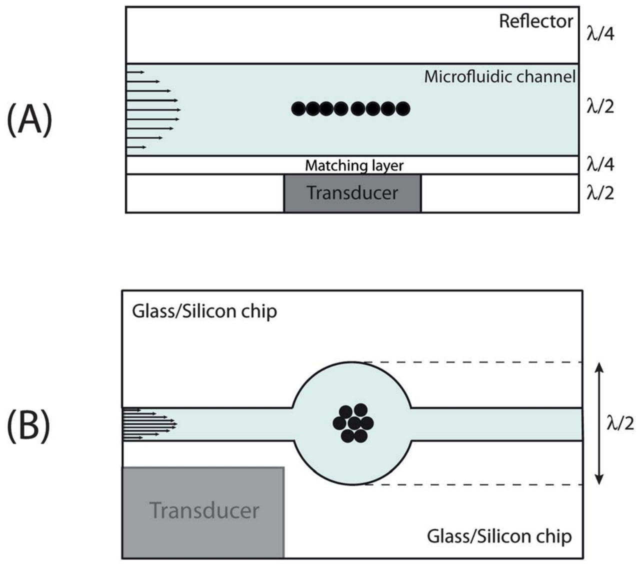

Fig. 8 (A) shows a side-view drawing of a trapping system using a localized acoustic field from a small transducer. The transducer is coupled to a microfluidic channel through a matching layer and creates a local standing wave that will trap and hold objects. (B) shows a top-view of an alternative approach where a resonance cavity is designed in a sealed and bonded glass/silicon chip. An external transducer actuates the entire chip and a standing wave will be supported only in the resonance cavity where objects can be trapped.

designed to use either a highly localized acoustic field, Fig. 8A or create a localized resonance, Fig. 8B.

Since the active trapping area in an acoustic trap using a transducer that creates a localized field near the transducer is essentially decided by the area of the transducer, it is generally easier to trap larger number of objects than in the resonance cavity approach. It is also usually easier to design an efficient system as it follows the same design rules as a layered resonator. However, these systems are typically harder to integrate with other lab on a chip systems and more difficult to scale up as they tend to rely on manually assembled transducers. An example of particles trapped over a localized transducer can be seen in Fig. 9.

The resonance cavity approach is on the other hand easy to scale up as it is fabricated using standard photolithography and etching. Although the trapping capacity of an individual cavity may be smaller than that of a localized transducer, it is easy to compensate for this by creating an array of cavities. The main disadvantage here is the difficulty in predicting the behavior of the system as the entire chip is actuated and can support many modes that might interfere with each other.

Examples of trapping systems with localized acoustic fields include designs with miniature transducers coupled directly to a microfluidic channel[31,32] and designs that use matching layers to couple transducers into flow systems.[33–36] Spengler *et al.* originally used a transducer with a rubber gasket and a stainless steel container around it to create a resonance cavity for trapping.[34] The system was later changed to include a stainless steel matching layer and a single inlet and outlet to allow for cell loading.[33] Lilliehorn *et al.* and Evander *et al.* used a printed circuit board (PCB) with miniature transducers that compose the bottom of a fluidic channel when a reflector lid with an etched channel was placed on top of the PCB.[31,32] This system was modified by Hammarström *et al.* to use a capillary as a fluidic channel rather than the etched channel lid, creating a more robust system that used disposable fluidic parts.[36]

The alternative approach, to obtain a localized resonance, is usually achieved by designing a resonance cavity that will support a standing wave when the entire chip is actuated,[37–40] see Fig. 10. A common approach used is to create a silicon layer with fluidic channels linked to a resonance cavity using DRIE and then anodically bond a glass lid to the chip. Manneberg *et al.* created a resonance cavity that allowed for three dimensional, selective standing waves using external transducers[40] and Vanherberghen *et al.* recently demonstrated a trapping cavity array that was used for microscopy studies of cells.[39]

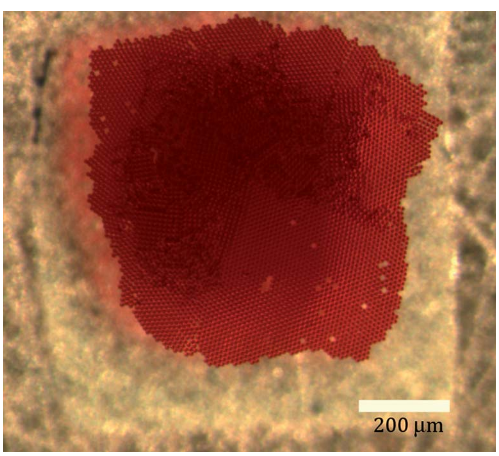

Fig. 9 10 μm polystyrene particles suspended in a flow above a square transducer. Courtesy of Björn Hammarström, Lund University.

Capillaries

The use of readily available fluidic components is of special importance when working with very sensitive detection methods or potentially hazardous samples and many times a disposable component is a prerequisite. In this perspective capillaries were successfully used in early approaches both for trapping and acoustophoresis. Capillaries or cuvettes have well defined dimensions, known surface properties (typically glass) and are available without the need of microfabrication or bonding.

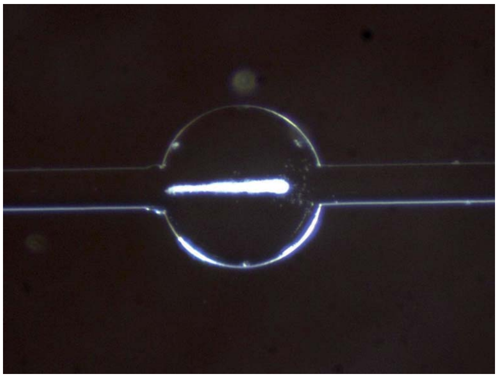

Fig. 10 A cluster of polyamide particles that are trapped in a resonance cavity and held against a flow in an all glass chip fabricated resonator, actuated at 2 MHz.

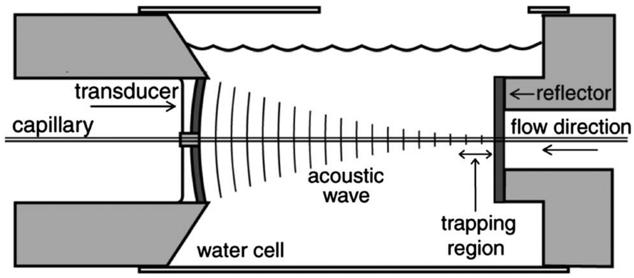

Fig. 11 A schematic drawing showing how a focused transducer and a continuous flow capillary were used for trapping. Reprinted with permission from ref. 42 Copyright 2001, American Institute of Physics.

Tilley *et al.* did an early experiment in 1987 using a capillary for studies of induced erythrocyte adhesion.[41] The system used a cuvette as a sample reservoir and the sample was drawn into the capillary by capillary forces. The cuvette rested on a 1 MHz transducer that created a standing wave in the direction of the capillary and aggregated the erythrocytes in order for them to be studied by microscopy. Wiklund *et al.* improved the capillary approach further by reducing the capillary dimensions significantly and designing an 8.5 MHz focused transducer and a reflector that allowed for a quartz capillary to pass through the centre of both units,[42] see Fig. 11. The entire assembly with capillary, transducer and reflector was immersed in a water bath to ensure that the acoustic waves from the focused transducer entered the capillary. This way a flow-through system was created that could trap and hold 3–4 μm particles. The system was later combined with capillary electrophoresis to investigate the possibilities of increasing the limit of detection by selective trapping of particles in an immunoagglutination assay.[43]

Goddard *et al.* used a capillary to create a continuous flow particle concentrator that was later used to improve the accuracy of a flow cytometer system.[44–46] A glass capillary with an inner diameter of 1.9 mm was actuated at around 460 kHz to focus 10 μm particles into a narrow band that would decrease the spread in the scattered laser signal as well as remove the need of a particle focusing sheath fluid, see Fig. 12. The transducer was applied using gel to the side of the capillary. To maximize the acoustic energy input into the system they used an identical transducer on the other side of the capillary as a sensor. The system was driven at the frequency that maximized the amplitude of the detected signal at the sensor transducer. By using this system, they could track the resonance and compensate for any drift that might occur.

Hammarström *et al.* recently presented a PCB-platform with miniature transducers coupled to a rectangular borosilicate capillary through a thin glycerol layer.[36] This way a localized standing wave for trapping could be created in the capillary while still having a disposable microfluidic component that could easily be changed between assays. The system was used for creating a trapping pipette which, when coupled with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), could be used for the study and drug partitioning in minute cell populations.[47]

Actuation

Transducer coupling

The coupling medium between the transducer and the resonator chip is crucial in deciding the efficiency of energy transfer to your resonator. Effects of the bonding/coupling layer was investigated by Hill *et al.*[48] using a transducer and an impedance transfer model. They concluded that the thin layer of adhesive used to couple the transducer only has a relatively small effect on the modelled electrical characteristics—as long as the layer was thin and "well bonded". Well bonded in this sense

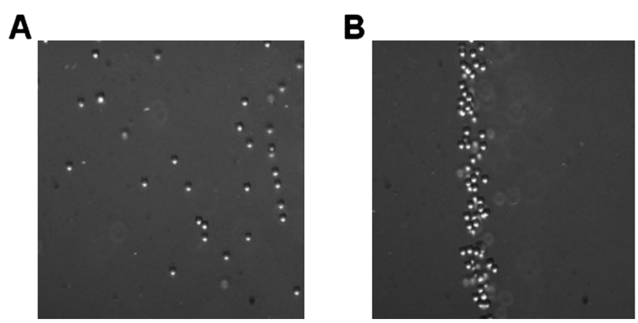

Fig. 12 (A) shows unfocused 10 μm polystyrene beads flowing through a glass capillary (I.D. 1.9 mm) at a speed of 5 mm s^{-1}. The capillary is actuated using a 417 kHz PZT attached to the outer wall of the capillary and aligns the particles as can be seen in (B). Reprinted with permission from Goddard *et al.*[45] Copyright 2006, John Wiley and Sons.

means no air voids between the transducer and the chip as they will act as a total reflector and reduce acoustic energy transfer to the chip. Most commonly the transducer is bonded to the resonator chip by *e.g.* epoxy or cyanoacrylate glues. Hard glues provide a better coupling as compared to *e.g.* silicone rubber based bonding layers.

If a system in which the transducer can be decoupled from the resonator chip is desired, the use of a hydrogel or glycerol as a coupling layer between the transducer and the chip can be employed,[49] much like the current practice in diagnostic ultrasound. Since the bond in this case is viscous, the transducer must be clamped to the chip to retain a constant pressure in the bond and the clamping must be spatially fixed to prevent the sandwich structure from sliding. Coupling of the transducer using a hydrogel is a convenient method when evaluating different resonator set ups and thus allows for rapid reconfiguration of the acoustic system. When using harder glues *e.g.* cyanoacrylate glues, they can be dissolved/softened in acetone overnight, enabling remounting or change of the transducer on the resonator.

Some publications describe systems where the transducers have been incorporated in the microchannel as one of the resonator cavity walls, delivering ultrasound directly in contact with the fluid.[31,50–52] This alleviates any undesired effects that are linked to a variance in the coupling layer. A side effect, however, is that the acoustic interference patterns from the transducer become more prominent. A benefit, however, is that the lateral gradients that arise in such a system can be utilised in creating strong acoustic trapping systems.[50]

Rather than mounting the transducer directly under the trapping zone, Haake and Dual have shown how surface waves can be used to perform two-dimensional particle manipulation.[53] By gluing shear transducers to the sidewalls of a glass plate, it was possible to create standing flexural waves that will couple to the fluid underneath the glass plate and cause particles or cells to aggregate in lines or points.[54] By slowly changing the driving frequency it was also demonstrated that the nodes in the standing wave could be moved and that cells could also be transported in this fashion.

The location of the transducer in relation to the resonance cavity varies throughout the literature. Layered resonators are always constructed with the transducer in line with the fluid and the reflector. For transversal resonators, the most common positioning of the transducer is underneath the channel.[15,55,56] However, the transducer does not have to be located directly under the channel in order to generate a standing wave in the resonator. As long as the transducer delivers acoustic energy into the transversal resonator chip at a frequency that matches the resonator, a standing wave will form. Augustsson *et al.* demonstrated this when gluing a small transducer die at the side of the chip yet being able to actuate a long resonator channel.[57,58] However, as pointed out by Hagsäter *et al.*, the obtained resonance pattern is dependent on the position of the transducer indicating that different system resonance modes are being actuated in relation to the spatial location of the transducer.[37] Yet, the chip can be actuated from any transducer position although some positions will be more efficient than others in terms of transmitted acoustic energy and hence heat generation. Wiklund *et al.* introduced a slightly different approach to the transducer coupling, by the use of metal wedges glued as the coupling medium between the transducer and chip. The wedges work as refractive elements in coupling the acoustic energy into the resonator structure.[59,60] One particular reason for this design was the need for optical access across the flow channel and still have an actuation mode that translates particles in the plane of the chip, similar to the transversal mode of operation.

As losses in the piezo electric transducer are the primary source of heating to the acoustophoretic system, the coupling layer can be designed to work as a heat sink in order to prevent an increase of temperature in the flow channel and thus a drift in resonance frequency. As an example, at a resonance of 2 MHz, an increase from 25 °C to 30 °C in water results in resonance frequency increase of 0.8% (~17 kHz). This could be sufficient to drive the system off resonance or at least weaken the magnitude of the standing wave considerably. It is therefore important to avoid temperature variations during operation and measures should be taken to counteract them by careful design or heat sinks, or integrated temperature control.

The intrinsic heat generation in the piezo transducer can be used to elevate the operating temperature of the microfluidic system. Evander *et al.* demonstrated that temperature control of an acoustic trap can be accomplished by calibrating the operating temperature at different actuation voltages in an acoustic trap.[51] By this means a temperature control of the trap was accomplished such that culturing and proliferation of yeast cells could be performed at 30 °C over an extended period of time. An optional approach was proposed by Wiklund where a heating fan was linked to the microsystem, enabling controlled temperature elevation of the system independent of the driving of the piezo actuator.[61] More recently Augustsson *et al.* demonstrated the profound impact that temperature changes actually have on acoustic resonators and also proposed a Peltier based feedback system to fully control system temperature independent of the driving conditions of the actuator.[62]

Aluminium offers good heat transporting capabilities and has also good acoustic properties. By adding a block of aluminium between the transducer and the chip, an acoustophoretic microchip can be operated at high driving voltages without getting overheated.[23] However, it is important to match the thickness of the aluminium block to a multiple of half a wavelength such that a resonance in the block itself is induced. This ensures that the acoustic energy delivered to the microchip is relatively high yet at moderate actuation amplitudes. An example of this is given below where a silicon chip with eight parallel channels was used together with aluminium coupling blocks of different thicknesses, *D*, to illustrate the importance of wavelength matched distance, Fig. 13a. Each channel width was matched to a half wavelength resonance at an actuation frequency of 2 MHz. As the system was actuated the microparticles passing through the channels in the chip were focused in the channel centre and at the channel trifurcation outlet the percentage of particles being collected in the central outlet served as a measure of the acoustic focusing efficiency. As seen in Fig. 13b,

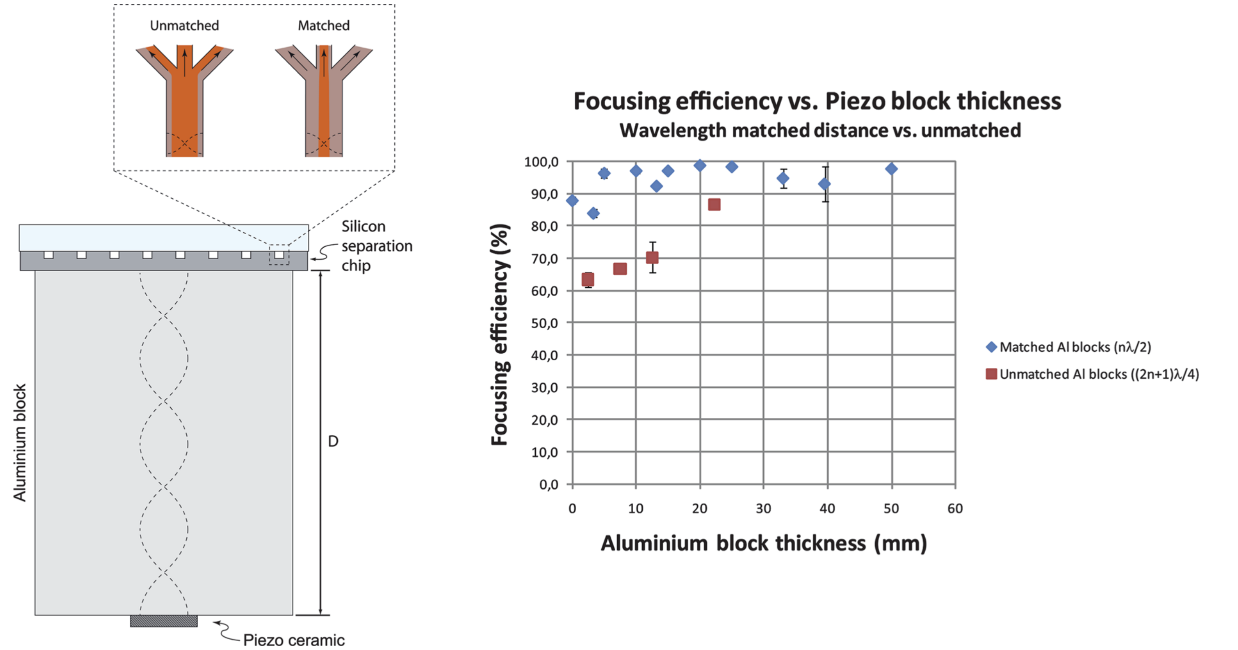

Fig. 13 (a) A silicon multi-channel chip with an aluminium block in between the transducer and separation chip which acts as a heat sink. (b) Figure showing the importance of having half wavelength matched coupling layers. Unmatched aluminium blocks (quarter wavelength matched) reduce the amount of acoustic energy transferred into the channel and affect the separation efficiency negatively.

a focusing chip with aluminium block distances that are unmatched, $(2n + 1)\lambda/4$, show a significantly lower focusing efficiency as compared to the matched blocks under identical power input conditions.

Coupled resonance modes

When designing acoustic resonators that are composed of several building blocks each of which displays individual acoustic resonances, undesired results may appear at the system level. The complete acoustic system with the actuator coupled to the resonator cavity may not display a resonance at the targeted frequency. A very good example of this was presented by Hill *et al.*,[48] where a layered resonator was designed to have a half wavelength resonance at 3 MHz, which was coupled to a piezo transducer bonded to a matching layer with a combined resonance frequency of 3 MHz. The combined resonance of the two parts did however not resonate at 3 MHz. Instead a frequency split was observed and two resonances appeared at about 2.9 MHz and 3.1 MHz. By choosing the transducer resonance and the design of the resonator cavity such that they have slightly mismatched resonances, a more stable and robust acoustic system is accomplished.

Electrode and transducer modifications

Modifications of the standard piezoelectric transducers, *e.g.* by modifying the electrode geometry, can make a big difference in the output. Neild *et al.* showed that by creating "strip electrodes" they were able to increase the number of resonance modes in a transducer and thereby actuate harmonics in their resonator.[63] In the example in Fig. 14, orthogonal strip electrodes were used to generate two independent pressure fields in a chamber. By superimposing the fields, a pressure distribution could be created in a fluid chamber that positioned cells in a 2D-array format.[64]

Focused transducers

Focused transducers have mainly been used in acoustic trapping systems where they help in creating the large acoustic gradient that is needed to trap objects.

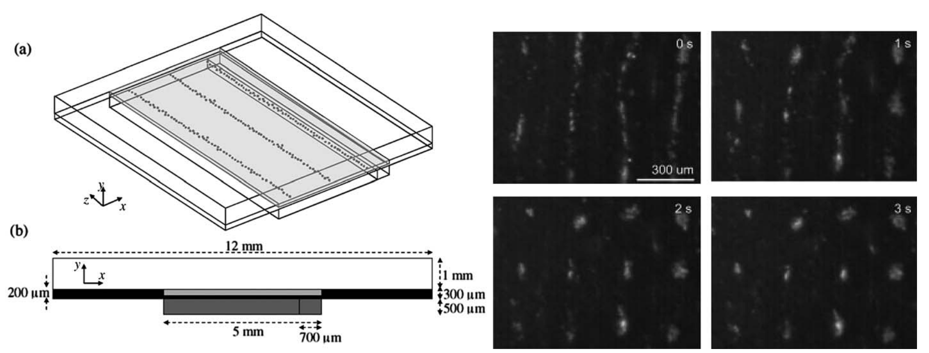

Fig. 14 Left: 700 µm strip electrode on a 5 mm wide piezo-electric transducer. Reprinted from Neild *et al.*[63] with permission from Elsevier. Right: 2D-positioning of cells in a fluid chamber actuated by a single transducer with orthogonal strip electrodes.[64] Copyright 2007, John Wiley and Sons.

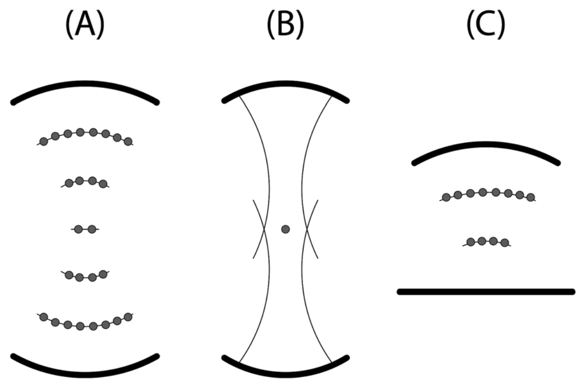

Fig. 15 Three different approaches for using a focused transducer when performing particle trapping. In (A), two focused transducers are aimed at each other and create a standing wave where particles can be trapped. (B) uses the pressure gradient that is created when two transducers are focused with a short distance between the focal points to trap objects. In (C), a hemispheric standing wave is created by having a planar reflector closer than one radius of curvature from the transducer.

They have been used in two different configurations, either with two focused transducers facing each other or a single focused transducer aimed at a reflector, see Fig. 15. In the first case, you can either form a standing wave between the transducers[65] or use the pressure gradients to create a potential well between the transducers.[66] In the second case, a hemispheric standing wave can be created that allows for object trapping in several nodes.[42,43,67]

Yasuda *et al.* envisioned a device consisting of phased arrays of ultrasonic elements in different configurations in a US patent with a priority date of 1992.[11] The phased arrays could focus the ultrasonic waves at an arbitrary position and create gradients that could be used for focusing, trapping and moving of particles.

Conclusions

When designing an acoustic manipulation system, the size range of the particles to be manipulated has to be taken into account. Smaller particles (1–3 μm) will require a higher operating frequency than larger particles to achieve the same radiation force. The frequency and the desired number of pressure nodes will in turn determine the dimensions of the acoustic resonator. Typical frequencies used are 1–10 MHz resulting in resonator dimensions of 750 μm to 75 μm when operated at a half wavelength resonance.

There are several suitable materials for acoustic applications although the choice depends on the resonator design. Transversal resonators are preferably fabricated from materials with high characteristic acoustic impedance such as metals, glass and silicon. The latter two being most commonly used as microfabrication techniques enable precise and reproducible structures in the desired size range. A layered resonator can also be constructed from high acoustic impedance materials although they could benefit from polymers even though polymers attenuate ultrasound. Polymers are also successfully used as microfluidic channels in SSAW devices.

Finally, to ensure a high Q-value in the system, a well bonded chip, matched reflection and matching layers and a thin coupling layer are required.

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