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# Microscale Acoustofluidics

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## Synonyms

Acoustic separation; Acoustophoresis; Position manipulation of particles by ultrasonic fields; Ultrasonic particle separation

## Definition

Microscale acoustofluidics defines the use of ultrasonic waves onto a fluid inside a microchannel. The acoustic waves are imposed on the fluid through excitation of the microchannel walls with an actuator. Acoustofluidics in microchannels is commonly used for moving and manipulating microparticles in a microchannel.

## Overview

Separation of cells has several applications in medicine. In apheresis applications, certain types of cells in blood are separated from the remaining cells such as in leukapheresis, plateletpheresis, erythrocytapheresis, and plasmapheresis. The general methods used for separation of cell types are filtration which relies on separation due to size differences and the use of centrifugation which is separation through density differences. In order to prevent clogging of the filter, the filter is rinsed with a fluid flowing at a high flow rate. The generated shear forces clean the filter; however, it may also damage or activate certain properties of the cells (such as clotting due to platelet activation). Similar shear forces are generated in centrifugation

methods as well. Use of acoustophoresis relies on difference in physical and acoustic properties of cells (such as speed of sound, density, and size), and it does not generate high shear forces; hence the viability of cells is not affected. Therefore the use of ultrasonic standing waves is a candidate technology that may replace filtration and centrifugation cell separation methods in the future.

Microparticles can be manipulated within a microchannel by acoustic waves imposed on the fluid through excitation of the microchannel walls with an actuator. The microparticles in the channel are moved across the cross section by acoustic radiation force which has a varying magnitude throughout the cross section of the channel. The distribution of the acoustic radiation force is determined by the acoustic mode shape present in the microchannel. The acoustic radiation force acts on the particle in such a way that the microparticles are moved to the nodal points of the acoustic mode shape. Therefore, the microparticle in a microchannel can be moved to nodal points of the acoustic mode shape which enables manipulating the positions of the microparticles. Acoustic radiation force magnitude also depends on density, size, and the acoustic properties of the microparticles. The differences of microparticles in these properties result in different forces on each cell which in return determine their location at the channel cross section at a certain time. Therefore, over time the microparticles end up at different locations along the cross section of the channel which results in separation of microparticles. The upside of this method is that it does not cause high shear stresses on the cells and the Joule heating problem of the electrical methods does not exist for ultrasonic separation process. Unlike electrokinetic, magnetic, and optical methods, acoustic retardation force is effective along the entire channel where the ultrasonic waves are present rather than a confined region. This aspect is an advantage for high-throughput applications.

## Basic Methodology

In this part, mathematical modeling of the acoustic radiation force acting on particles in a microchannel is presented. The solution methods for determining the generated radiation force and determining the particle response are introduced.

The starting equations for acoustics problems are the continuity equation and the Navier-Stokes equations for compressible medium. The continuity equation for compressible medium is as follows:

$$\frac{\partial \rho}{\partial t} = -\nabla .(\rho \mathbf{v})$$

where $\rho$ is the density of the medium and $v$ is the acoustic velocity of the particles. The Navier-Stokes equation of compressible medium is as follows (body forces are neglected):

$$\rho \frac{\partial \mathbf{v}}{\partial t} = -\nabla p + \eta \nabla^2 v + \mu \nabla (\nabla .\mathbf{v}) + \rho (\mathbf{v}\nabla)\mathbf{v}$$

where $p$ is the pressure and $\mu$ is a constant related to viscosity of the fluid. In acoustics rather than the absolute value of density and the pressure, small variations (due to acoustic waves) of density and pressure from the mean values are important. These small variations are assumed to be harmonic in nature:

$$p = p_0 + p_1 \text{ and } \rho = \rho_0 + \rho_1$$

where $p_1$ and $\rho_1$ are small harmonic perturbations around the absolute static values of pressure and density ($p_0$ and $\rho_0$). If the purpose is to find the fluctuations in pressure and density due to acoustic waves, one can substitute the pressure and density equations above into continuity and Navier-Stokes equations and solve for acoustic parameters. However, in the calculation of radiation forces, it is required to integrate the pressure value around one cycle of perturbation. Since perturbations are harmonic, the integration around one cycle results in zero acoustic radiation force. Therefore, for the evaluation of harmonic forces, using first-order perturbations are not sufficient. Therefore, second-order variations are taken into account where the pressure and density variations are given as

$$p = p_0 + p_1 + p_2, \rho$$
$$= \rho_0 + \rho_1 + \rho_2 \text{ and } v = v_1 + v_2$$

If the above two equations are substituted in the continuity and Navier-Stokes equations and inviscid flow is assumed, after several steps of derivations, time-averaged second-order variation is given as follows [1]:

$$\langle p_2 \rangle = \frac{1}{2\rho_0 c_a^2} \langle p_1^2 \rangle - \frac{1}{2} \rho_0 \langle |\mathbf{v_1}|^2 \rangle$$

In the above equation $<\cdot>$ denotes time average over one cycle of acoustic excitation. If the integral of the pressure is taken around a spherical region and the momentum flux term is added, the radiated force due to acoustic field onto a particle in the acoustic field becomes

$$\mathbf{F}_{rad} = \int_{\partial\Omega} \left\{ \left[ \frac{1}{2\rho_0 c_a^2} \langle p_1^2 \rangle - \frac{1}{2} \rho_0 \langle |\mathbf{v_1}|\rangle \right] \mathbf{n} + \rho_0 \langle (\mathbf{n}.\mathbf{v}_1)\mathbf{v}_1 \rangle \right\} da$$

where $\partial\Omega$ is the area over the sphere and "a" is radius of the sphere. In order to evaluate the values of the above radiated force expression, one needs to evaluate this integral on the surface boundaries of each particle in the field. In order to estimate the radiated acoustic force expression, one can employ different methodologies. Here, three of these methods will be discussed.

The performance of a separation process can be assessed by using a computational model. To illustrate this, the performance of the device proposed by Petersson et al. [2] will be assessed with the current computational models. On the aforementioned setup, separation of polystyrene beads with three different diameters was aimed (diameters of 3, 7, and 10 μ). Particles are released from inlets 1 and 2 with uniform distribution along the cross sections of the inlets. After the inlets merge on to the main channel, the main channel continues for 30 mm, and the separated particles exit through outlets 1, 2, and 3 according to their sizes (10 mm exits from outlet 1, 7 mm diameter exits from outlet 2, and 3 mm exits from outlet 3). The ultrasonic excitation was applied only to 20 mm portion of the 30 mm channel by means of a piezoelectric actuator.

In order to solve this problem as well as acoustic field calculation, flow field calculations should be performed so that the fluid velocities are known at each location in the channel. Velocity field data is calculated from CFD solution with inlet and outlet flow rates as the input to the CFD model. The flow velocities in x, y, and z directions for each location in the channel are the output from the CFD model. These velocities are then fed into acoustic field models. The assumptions in the model are that the fluid flow is not affected from the particles and that there is no interaction between particles. The width of the channel is 370 μ and the depth of the channel is 125 μ (towards the plane of the paper); hence it is assumed that the acoustic field is uniform along the depth of the channel. Under these assumptions using the acoustic domain solution, the forces on each particle are evaluated. The first approach described below is estimation of the ultrasonic radiation forces using an analytical approach.

## Simulation of Particle Trajectory-Analytical Approach

The integral equation for radiated force can be evaluated for a spherical particle with the assumptions that the combination of scattered and the incident field around the particle can be represented by assuming the particle as both a monopole and a dipole acoustical source. The radiated field due to these sources and a standing acoustic wave in a rectangular channel is derived as below:

$$\mathbf{F}_y^{rad} = 4\pi a^2 (ka) E_{ac} \Phi \sin (2ky)$$

$$\Phi = \frac{\rho_p + 2/3(\rho_p - \rho_0)}{2\rho_p + \rho_0} - \frac{\rho_0 c_a^2}{3\rho_p c_p^2}$$

where $\Phi$ is the acoustophoretic contrast factor, $E_{ac}$ is the acoustic energy density, and $ka = 2\pi a/\lambda$ is the size to wavelength ratio.

Balancing the viscous Stokes drag force ($\mathbf{F}_y^{drag}$) with acoustophoretic force ($\mathbf{F}_y^{rad}$) results
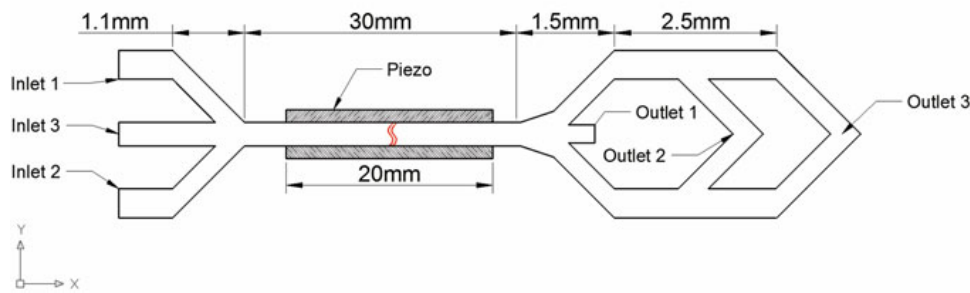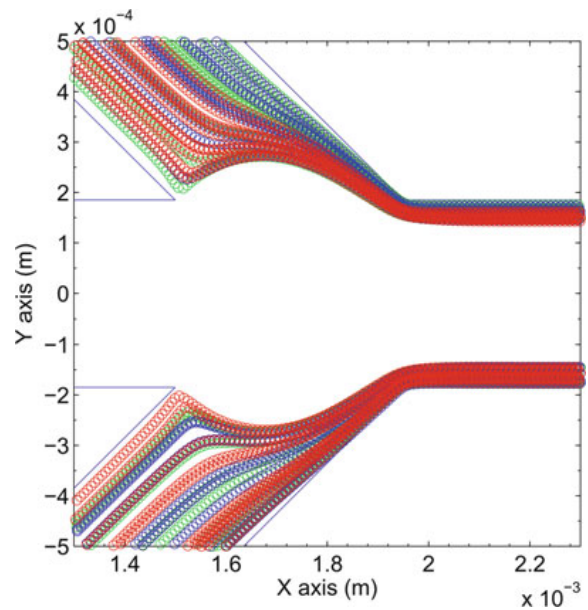
**Microscale Acoustofluidics, Fig. 1** Channel geometry

$$6\pi\eta a \frac{dy}{dt} = 4\pi a^2 (ka) E_{\mathrm{ac}} \Phi \sin (2ky)$$

$$y(t) = \frac{1}{k} \arctan \left\{ \tan [ky(0)] \exp \left[ \frac{4\Phi}{9} (ka)^2 \frac{E_{\mathrm{ac}}}{\eta} t \right] \right\}$$

Velocity field data is taken from CFD solution and used for calculating path of particles and determining which outlet they exit through. It is assumed that there are no interaction between particles, no distortion in the acoustic field, and no velocity in the z direction.

Channel geometry is based on study of Petersson et al. [2]; in that study, three different groups of microscale beads are separated. Microscale beads have 3, 7, and 10 μm diameter and 5 %, 10 %, and 15 % standard distributions in diameter, respectively. Channel geometry in Fig. 1 is used for modeling.

Figure 2 shows the microparticles after being released from the inlet before going into the channel where ultrasonic waves are applied. Red points represent nominally 10 μ particles; blue, nominally 7 μ particles; and green, 3 μ particles. Figure 2 shows that initially all the beads are in mixed state. After passing through the separation channel and being exposed to ultrasonic waves for 20 mm, it can be seen from Fig. 3 that the majority of the red particles are passing through outlet 1, and blue beads and the green particles are going away from the first outlet towards second and third outlets. Figure 3 shows that majority of the green particles are close to the wall which would have them end up in outlet three, whereas the blue particles are at a location which is closer to outlet two. The results of the simulation were percentages of



**Microscale Acoustofluidics, Fig. 2** Junction of inlets 1, 2, and 3

particles, and their corresponding outlets will be presented in the next section.

Analytical method is faster than finite element method and reasonably accurate. Also, it can solve too many particles and give more accurate results for models that contain distributions.

However, it is only applicable to uniform rectangular cross-shaped channels. For high-concentrated particle solutions, analytical method may give inaccurate results because distortion in acoustic field would affect the results.

## Simulation of Particle Trajectory-Finite Element Approach

Finite element method can be used to simulate the ultrasonic radiation force on the particle and to simulate how particle moves inside the channel

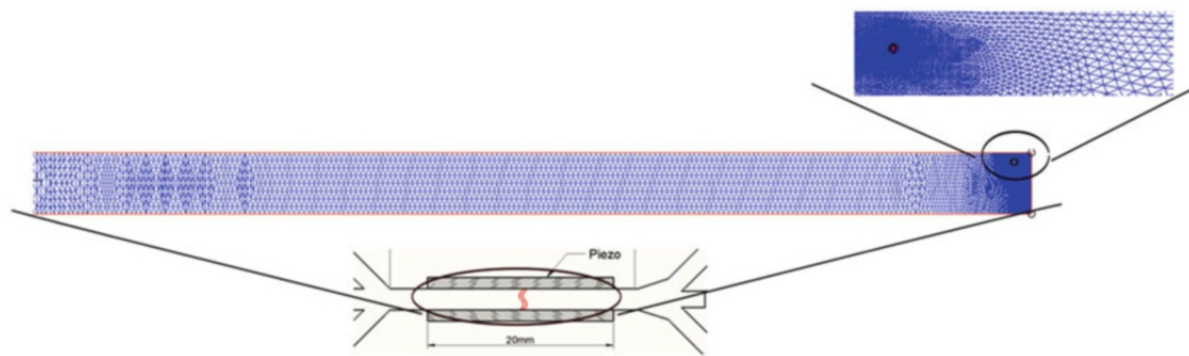**Microscale Acoustofluidics, Fig. 3** End of main channel
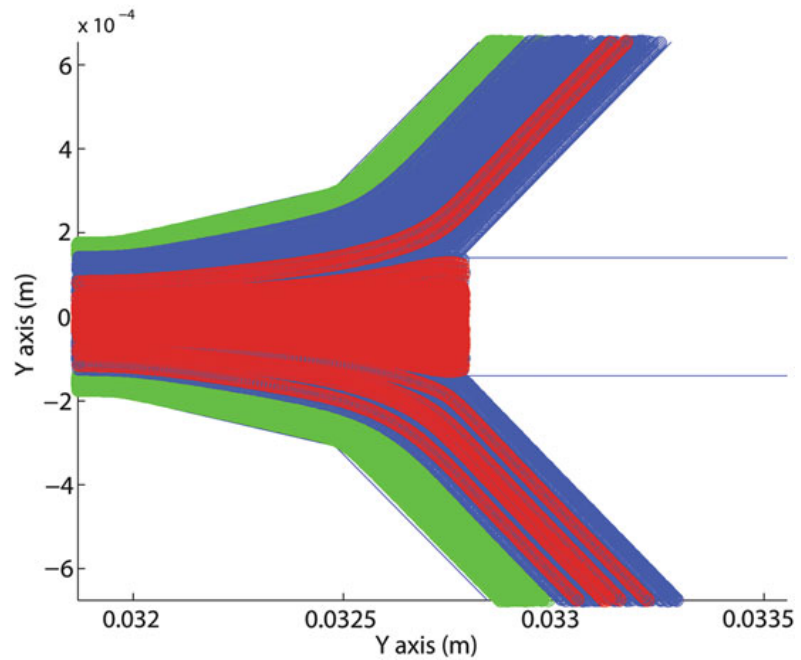


**Microscale Acoustofluidics, Fig. 4** Acoustic pressure distribution in the channel because of piezoelectric excitation on the wall

under this ultrasonic radiation force. Initially, the channel geometry is drawn, and the CFD analysis should be completed with the flow rates from each inlet and outlet as the input to the CFD analysis. Once the CFD analysis is completed, the next step is to start the beads at random locations of the inlet and simulate the flow of the beads using the velocity components computed from the CFD analysis. Once the beads reach the region where ultrasonic excitation exists, the field created by the wall movement is calculated using a finite element program, and the acoustic pressure distribution is calculated. The solution in the acoustic domain is only performed at the location where the piezoelectric material is located. It can be seen in Fig. 4 that the ultrasonic excitation does not propagate towards the other parts of the channel but rather is local to region where piezoelectric material excites the channel (the region shown in color).

The ultrasonic radiation force exerted on the particle is calculated at each time step through evaluation of integral equation for radiated force. It should be noted that the acoustic pressure and velocities are calculated using the finite element code. These solved values of acoustic velocity and acoustic pressure is substituted in the integral equation. At each time step, velocities imparted on the particle due to fluid motion are also taken into account through CFD analysis. Therefore, at each time step (which is around 0.5–5 ms), the new location of the particle is calculated and the particle is moved. At the new time step, geometry with the new particle location is meshed and solved again. Once the particle leaves the region where the ultrasonic waves are active, then CFD analysis determines the path of the particles.

Like the analytical approach, particle diameters have a random (normal) distribution, and starting locations of the beads have uniform

**Microscale Acoustofluidics, Fig. 5** The meshed region where ultrasonic waves are present

distributions. One of the main challenges of the finite element model approach is the computational time required for the analysis. The geometry (Fig. 1) is composed of a long narrow channel where a small particle is in the channel. Due to small size of the bead (3–10 μ), there is a need for small mesh size around the particle; however, the length of the channel is three orders of magnitude larger than the particle. This causes difficulty in meshing. Figure 5 shows a particle just before it left the ultrasonically excited region. It can be observed that the region around the particle is extremely densely meshed, and as the particle moves, this densely meshed region moves with it. Therefore, the method of boundary element analysis has clear advantages since the inside of the domain need not to be meshed.

For the simulation of particle trajectory, with the presence of the particles is a computationally expensive process, since the particles are moving within a microchannel, which requires remeshing of the computational domain. One alternative approach could be the implementation of boundary element method (BEM). BEM has a unique advantage over the conventional PDE solution procedures as the meshes are generated only on the boundaries of the computational domain. Therefore, the motion of the particles within the microchannel requires the movement of the meshes located on the particle.

## Key Research Findings

There are host of studies that use ultrasonic waves in cell/particle manipulation and separation. However, most of these studies are application oriented, and the numerical modeling efforts for this method are rather limited.

## Experimental Study Findings

Particle/cell manipulation aims to position the particles/cells at a certain location inside the channel. The purpose is not necessarily to separate these cells but to control their location. Controlling the location of cells is particularly important for cell washing and cell concentration purposes. A common target in these studies is to position the cells in the pressure node locations. In several studies, the particles are positioned at pressure node locations successfully [3]. In a rather recent study, Glynne-Jones et al. [4] were able to position the microparticles to any location in the microchannel cross section by feeding a mode-switched signal to the piezoelectric element.

The positioning of cells in a microchannel is also studied. Several studies were able to move living cells to pressure nodes using ultrasonic waves. *Saccharomyces cerevisiae* and *Escherichia coli* (*E. coli*) cells are positioned to nodal locations [5]. In the study of Kapishnikov et al. [6], the blood cells from rabbits are separated from the blood plasma by positioning the cells in the pressure nodal location.

As the method started to mature, there have been more studies that aimed at separating the particles of different sizes or densities from each other as well as different types of cells from each other. The studies with particles aim at separating particles of different diameters from each other. In the study of Petersson et al. [2] microparticles

with diameters 2, 5, 8, and 10 μ were separated from each other. Also, in the study of Adam and Soh [7], an ultrasonic separation channel was designed that works with the principle of a band-pass filter. This setup successfully separated the beads 1, 3, 5, and 10 μ from each other.

The ultimate target of this research field is to be able to separate living cells from each other. In study of Petersson et al. [8], the lipid particles were tried to separate from erythrocytes. Also the same group tried to separate human blood cells from each other (red blood cells, white blood cells, and platelets) [2]. In other studies, different types of cell separations were targeted such as human sperm from egg cells [9].

From the findings of these studies, it is clear that position manipulation of cells and particles is successfully performed in microchannels. The separation of particles according their sizes is successfully performed in several studies as well. However, there are some difficulties when it comes to separation of cells from each other. If the cells are acoustically very different, the results seem to be more successful, but if the size, density, and speed of sound variations in a single-cell group are rather large, this poses a challenge for the separation process. The results can be improved if the sensitivity of the performance to the important parameters of ultrasonic separation is better understood. An effective way to understand these sensitivities may be by numerical modeling of the ultrasonic separation process. For the rest of the section, some results and findings of the ultrasonic separation process will be provided.

### Numerical Study Findings

For the system given in Fig. 1 using the analytical and finite element approaches, the performance of the system is evaluated. The methodologies used for these two methods are explained in the previous section. The results of these two simulation approaches will be compared to experimental results given in the work of Petersson et al. [2]. The simulation system is the replica of their experimental study performed with 3, 7, and 10 μ beads. The results of the experimental study are adopted from work of Petersson et al. [2] and

given in Fig. 6. The experimental results show successful separation of 10 μ particles and 3 μ particles, and 7 μ particles seem to mostly end up at the targeted outlet of 2. However, there is significant population of 7 μ beads at the unintended outlets of 1 and 3 (30 %). The results of the ultrasonic separation simulations using the analytical methods show similar estimations of separation as shown in Fig. 7. The main difference with the experiments is in the 3 μ particle results. The simulations show very successful separation of 3 μ beads; however, in the experiment, only 80 % of the 3 μ beads ended up in the targeted outlet of 3.

Figure 8 shows that the results for the finite element simulations are also similar and the results for separation performance of 3 μ particles are better than the experimental results. On the other hand, the other estimations for the bead locations are accurate, and the general trends of separation performance are successfully captured. Therefore, it seems that the simulation tools can be used effectively to estimate the response of the ultrasonic separation system. These numerical modeling tools give possibility to numerically try and optimize the sizes and the low rates of the separation systems before experimental tests.

The differences between the analytical model and the finite element model results are not significant, and in terms of computation time and the initial setup of the numerical model, the analytical method has clear advantages. However, there are certain cases where finite element modeling may have advantages over the analytical modeling method. If the concentrations of the beads are so high that the acoustic field inside the channel is significantly changed by the particles, then this change in the acoustic field can only be captured by the finite element model. Also if the channel shape is not rectangular or it has a varying cross section over the length again, the finite element modeling is more advantages to be used compared to analytical method.

Another observation coming from the simulation is as follows: the positions of the particles at the beginning of main channel determine which channel they exit through. If a 7 μm bead enters

**Microscale Acoustofluidics,**
**Fig. 6** Bar plot of
experimental data
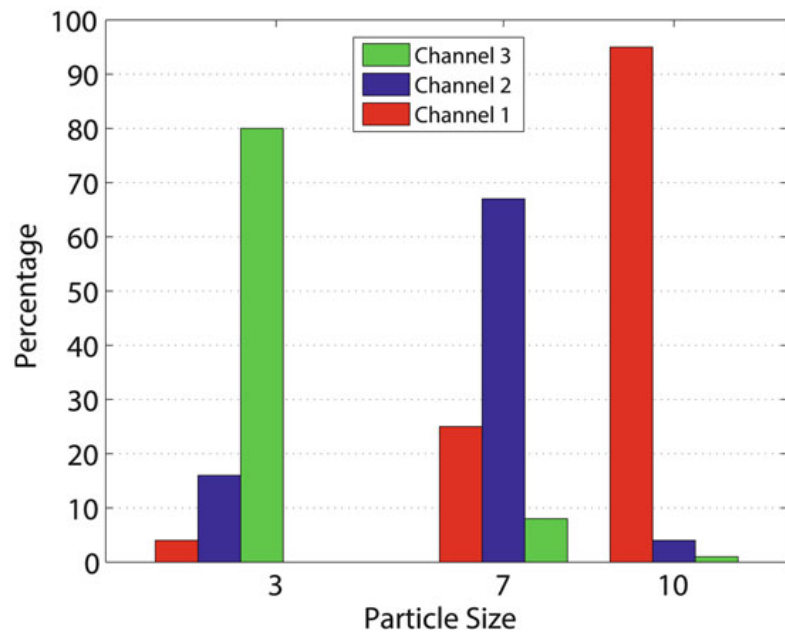


**Microscale**
**Acoustofluidics,**
**Fig. 7** Bar plot of
analytical modeling results
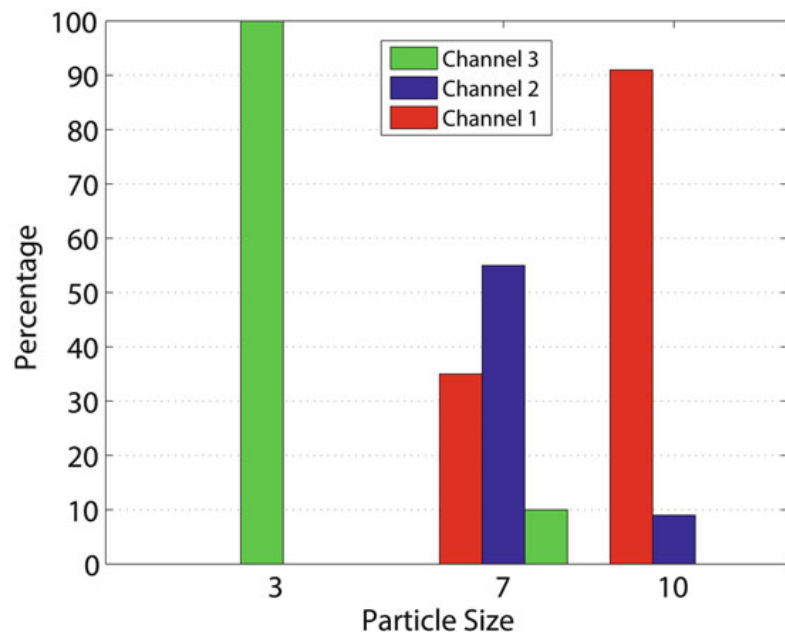


the channel close to channel's center and 10 μm bead enters close to wall, 7 μm bead can exit through outlet 1, while 10 μm bead can exit through outlet 2. This means that flow characteristics affect performance as much as acoustic characteristics. Velocity of the particle determines time period the particle is exposed to acoustic force. For example, if a particle is close to the top or bottom walls of the channel, it travels further in the transverse direction than an identical particle in the middle of the channel.

## Future Directions for Research

Acoustophoresis needs to be a process so robust and efficient that it can be used as an alternative safe (causing less shear on cells), economical, and fast (label-free) cell separation method for diagnostics and therapeutic medical applications. In order to increase the robustness of ultrasonic separation process, it is important that parameters that improve the performance are well understood and optimized. Numerical simulations

**Microscale Acoustofluidics, Fig. 8** Bar plot of finite element modeling results
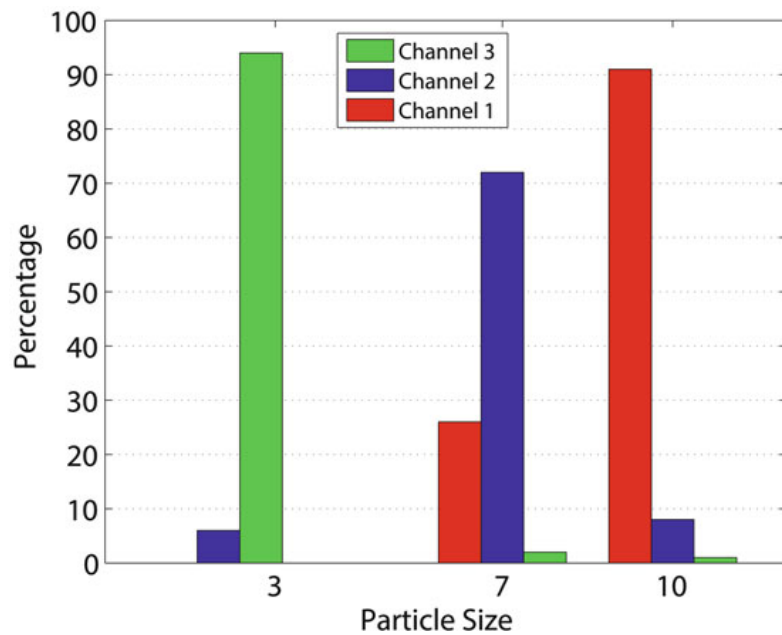


may serve as an efficient tool for this purpose. Accurate simulations are important to improve the performance of ultrasonic separation process, but accurate simulations generally mean high computation times (high mesh density and small time steps). In order to improve computation times, boundary element modeling approach can serve as an important tool. It does not suffer from domain meshing and does not have the limitations of analytical simulation methods (spherical geometry, rectangular geometry, etc.). Therefore, studies that model the acoustophoresis process with boundary element method is a future direction of research that needs to be explored.

One important parameter for successful simulation of acoustophoresis is to know acoustical properties of microparticles that are being fractionated. This is especially important if the acoustical properties (such as density, speed of sound, and compressibility) of microparticles are not known beforehand. Therefore, there is a need for a fast and reliable method that would identify the acoustic properties of the cells/particles that are aimed to be fractionated. Also during the simulations, it has been observed that parameters other than acoustical parameters play an important role in the performance of separation process. Fluid domain-related parameters affect the separation process. The simulations show that

depending on the particle starting position in the channel cross section, same particle under same ultrasonic field can end up in different outlets of the channel. Random parameter such as starting position in the cross section should be prevented from affecting the performance of the separation process. One possible way is to manipulate the positions of the particles/cells before they are separated according to their sizes. In other words, particles and cells are positioned to the pressure node locations which would prevent the variability of starting position. Once they all are at the predetermined position, then another frequency and a secondary piezoelectric material can be used for separation of these aligned cells/particles. This approach should result in more robust separation performance.

Another possible approach for future studies might be hybridization of the method with other cell separation methods. One good potential might be hybridization of dielectrophoretic method and acoustophoretic method. Dielectrophoresis is chosen for hybridization since this method is extensively studied and its separation performance can be improved by keeping the distance between the cells and the electrode (s) consistently close. In that sense, the positioning of cell can be achieved by positioning the cells towards a pressure node. As an example,

the separation of living cells from dead cells can be achieved by dielectrophoresis [10], and its performance can be improved by using acoustophoresis if the cells are positioned at certain location across the cross section. This approach can give the selectivity, robustness, and the throughput required from a cell separation device.

Using acoustophoretic methods in medical devices for therapies is possible if the throughput of acoustophoretic devices is increased. The most obvious methods for increasing throughput are to increase the flow rates or increase the number of separation channels. Due to several reasons, it is not possible to increase the flow rates orders of magnitudes (due to pressure drops across microchannel, excessive shear, etc.). However, it is possible to design multiple channels where there would be tens or hundreds of separation channel working in parallel. This parallel multiple designs will increase the throughput but significant amount of design and manufacturing effort is required to make it work. The important factors to look out for are manufacturability of this multiple channel design, excitation of all the channels with ultrasonic field, and fluid routing architecture for multiple channel design. However, if the above challenges are overcome, it would be an important step for a commercial diagnostic and therapeutic cell separation medical device that works with the principle of acoustophoresis.

## Cross-References

▶ Boundary-Element Method in Microfluidics
▶ Cell Sorting
▶ Lab-on-a-Chip Devices for Particle and Cell Separation
▶ Particle Manipulation Using Ultrasonic Fields
▶ Piezoelectric Materials for Microfluidics

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# Microscale Cooling Devices

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## Synonyms

Flow of coolants in micro-conduits; Microchannel heat sinks (MCHS); Micro-heat exchangers; Miniature heat-removal devices; Nanofluid flow in microchannels