USWNet 2012 Conference
September 21-22, Lund, Sweden

Abstracts & Scientific program
WELCOME TO USWnet 2012!

Welcome to the 10th Ultrasonic Standing Network Conference, USWNet 2012, to take place in Lund, Sweden, September 21-22.

USWNet stands for ‘Ultrasonic Standing Wave Network’ and was initiated in 2003 in the UK bringing together scientists with common interests in particle, cell and fluid movement and manipulation induced or controlled by ultrasonic standing wave vibrations.

The conference offers a premiere meeting place for all aspects on ultrasonic standing wave technology for particle and fluid manipulation ranging from fundamental theory and physics to applications.

Thomas Laurell
Chairman
USWNet Conference 2012
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Mauricio Hoyos¹, Angelica Castro¹, Wei Wang², Tom Mallouk²  
¹ Laboratoire de Physique et Mécanique des Milieux Hétérogènes, France; ² Pennsylvania State University, USA |
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Chair: Andreas Lenshof |
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Philipp Hahn, Jurg Dual; Institute of Mechanical Systems, Dept. of Mechanical and Process Eng, ETH Zurich, Switzerland |
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Kian-Meng Lim¹, Yang Liu²  
¹ Department of Mechanical Engineering, National University of Singapore; ² Sino-French Institute of Nuclear Engineering and Technology, Sun Yat-Sen University |
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Leah Johnson¹, Lu Gao², C. Wyatt Shields¹, Gabriel Lopez¹  
¹ Duke University, Department of Biomedical Engineering, USA; ² Duke University, Department of Mechanical Engineering, USA; ³ NSF Research Triangle Material Research Science and Engineering Center, USA |
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Dirk Möller, Nicolas Degen, Jürg Dual  
Institute of Mechanical Systems, Dept. of Mechanical and Process Eng., ETH Zurich, Switzerland |
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Massimiliano Rossi¹, Álvaro Marin¹, Per Augustsson², Rune Barnkob³, Peter B. Muller³, Thomas Laurell⁴, Henrik Bruus², Christian J. Kähler¹  
¹ Institute for Fluid Mechanics and Aerodynamics; Munich Bundeswehr University, Neubiberg, Germany; ² Department of Measurement Technology and Industrial Electrical Engineering, Lund University, Sweden; ³ Department of Micro- and Nanotechnology, Technical University of Denmark, DTU Nanotech, Denmark; ⁴ Department of Physics, Technical University of Denmark, Denmark |
Marco Travagliati¹, Marco Pagliazzi¹, Fabio Beltram¹  
¹ NEST, Scuola Normale Superiore; ² Center for Nanotechnology Innovation @ NEST, Istituto Italiano di Tecnologia; ³ NEST, Istituto Nanoscienze-CNR and Scuola Normale Superiore, Italy |
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Dinner Friday, September 21, 19.00

In 1830 the Academic Society was founded and the building, AF-Borgen, was finished in 1851. Today AF-borgen (The AF Castle), is a corporation. The Academic Society of Lund is the sole owner of all shares.

Meet colleagues and friends at Tegnérs Matsalar at 19.00. (AF-Borgen)

Tegnérs Matsalar (AF-Borgen)
Sandgatan 2, LUND
+46 46-13 13 33
Acoustic Focusing Flow Cytometry

Gregory Kaduchak and Michael D. Ward

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Introduction

Acoustic cytometry is a technology that replaces or partly replaces hydrodynamic focusing of cells or particles in flow cytometry with forces derived from acoustic radiation pressure. The ability to focus cells into a tight line without relying on hydrodynamic forces allows many possibilities outside the scope of conventional flow cytometry. Dilute samples can be processed quickly. Flow velocities can be varied allowing control of particle delivery parameters such as laser interrogation time and volumetric sample input rates.

Recently, Life Technologies developed a flow cytometer that directs particles into the laser interrogation region using acoustic radiation pressure. In this talk, the application of acoustic cytometry in flow cytometry systems from fundamental principles to its implementation will be presented. Data will be shown of the operational implementation of the acoustic focusing device that demonstrates its performance and repeatability. Experimental data demonstrating its ability to perform for complex biological assays will be given relative to standard hydrodynamic focusing systems.

Fig. 1. Acoustic Focusing Flow Cytometer.
A High Flow Rate Acoustic Focusing Capillary: Enhancing Flow Cytometry for Remote Plankton Monitoring

Daniel M. Kalb, Robert J. Olson, Heidi M. Sosik, Menake E. Piyasena, Steven W. Graves

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Introduction

Microbes account for most of the primary productivity and biomass in the ocean, and the structure of the microbial community determines in large part that of higher trophic levels. A fundamental understanding of the factors that regulate community structure requires detailed and sustained observations of the plankton. A fully automated submersible remote Flow Cytometer has been successfully deployed to actively characterize the large phytoplankton and microzooplanktonic organisms of coastal water. To effectively monitor the dilute oceanic phytoplankton population it is highly desirable to increase the throughput of the device from 250 \( \mu \text{L/min} \) at least 10 fold (greater than 2500 \( \mu \text{L/min} \)). Unfortunately, simply increasing the sample delivery rate widens the core to the point at which the cells are no longer in focus, while raising the sheath rate to increase the linear velocity will blur the images. Here we present our work to develop a high flow rate acoustic focusing capillary to pre-concentrate the cells prior to entering the imaging flow cell. This allows us to increase the particle analysis rates without increasing the linear velocity. Using 10 \( \mu \text{m} \) polystyrene beads as a model system, we achieve tight focusing at flow rates up to 10 mL per minute, while examining the 2D focusing efficiency of our 500 \( \mu \text{m} \) ID glass Capillary focusing system. These experimental results of the radial energy distribution within the capillary are being compared to refine and extend existing models.

Experiment

A single 1.5 MHz PZT bonded with superglue to our acoustic focusing glass capillary (5 mm ID x .25 mm Wall x 10 mm Length) is used to pre-concentrate the sample into the central node of the system. The standing wave is created by driving the PZT at its best half wavelength resonant frequency of 1.469 MHz. Resonance is maintained by manually monitoring the relative phase and amplitude of a second feedback PZT. An EM CCD imaging microscope and a 45-degree 5 mm mirror is used to monitor the 2D tightness of our acoustic focusing. The two fields of view, monitored to compare the relative efficiency of focusing in each dimension, are 90-degrees apart. The "Top" view is parallel to the PZT's plane of focusing while the "Side" view of the mirror image is perpendicular to the plane of the PZT.

Results

Using 10 \( \mu \text{m} \) polystyrene beads as a model system we are able to achieve tight focusing at flow rates up to 10,000 \( \mu \text{L/min} \), a 40-fold improvement in flow rate over hydrodynamic focusing. As expected, the voltage required to achieve focusing depends on the square root of the flow rate. The variation in performance between the top and side views leads us to the hypothesis that the energy density distribution in each
dimension is a function of the applied voltage. At low voltages we appear to be operating in an "under-driven" mode where most of the acoustic energy is propagated along the PZTs axis. As we increase the input voltage the efficiency of focusing in each dimension gradually becomes equal. We appear to be approaching an "overdriven" mode where we are physically vibrating the entire glass capillary equally in all dimensions. The model to describe these observations will be expanded and explained in more detail. At high voltages, driving the system in the "overdriven mode" we are able to achieve focusing far upstream, at least 75 mm, of the PZT. This observation reinforces the idea that at high voltages we are physically vibrating the entire glass capillary and suggests that we want to continue to use a long (100 mm plus) capillary in our final system. Using the same methodology, we will also discuss how we see a similar trend in a 1.033 MHz (.7 mm ID) square capillary system.

Figure 2. a) Voltage vs Flow Rate for both side and top views. At low voltages the side view (parallel to PZT propagation) concentrates at lower voltages. b) Energy Density Ratio of the top and side planes as a function of the side voltage. As the applied power increases the ratio approaches one.

Figure 3. a) At 150 µL/min for the side view we see how the focusing break up (peak width increases) as a function of Voltage at various distances upstream of the driving PZT. b) Normalized energy density required to focus beads as a function of position upstream of PZT.

Conclusion

In this work, we have shown that it is possible to tightly focus beads at high flow rates (10 ml/min) through a single node acoustic system. Additionally, the relative efficacy of focusing in 2D, parallel and perpendicular to the driving PZT, and far upstream (up to 75 mm) of the PZT was experimentally examined. Comparing these experiment results to our developing model will allow us to successfully maximize the efficiency of our system under the tight power and space constraints necessary for direct implementation into the submersible flow cytometer.

References

Improved Coulter Principle Cytometer Using 2D Acoustophoresis

Carl Grenvall\textsuperscript{1}, Christian Antfolk\textsuperscript{1}, Christian Zoffmann Bisgaard\textsuperscript{2}, Steen Kjær Andersen\textsuperscript{2} and Thomas Laurell\textsuperscript{1}

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Introduction

Here we present, for the first time, how acoustic forces can be used to drastically improve the performance of a microchip impedance flow cytometer (MIC) by aligning the sample particles, such as cells, in an upstream prefocusing zone of a microfluidic chip. This addresses known MIC sensitivity issues, due to varying particle positions in the flow channel, which other groups have also tried to solve using for example sheath flow alignment or intricate multilayer microchip structuring [1-4]. In comparison to these solutions, the acoustic approach allows for less complicated chip fabrication and can be used for on-chip sample preparation in addition to the prefocusing of the target particles [5]. Our proof-of-concept data, using polystyrene beads and red blood cells (RBC), show that on-chip acoustophoretic prefocusing of particles can be used to improve MIC performance.

Experiment

The microfluidic glass chip was designed in Lund and fabricated by Micronit Microfluidics BV using two step wet etching with planar platinum electrodes patterned across a narrow flow channel (35x80 µm) in order to allow impedance spectroscopy measurements, Figure 1.

![Figure 1: View of the microfluidic glass chip with patterned electrodes and fluidic connections (a) with the impedance microscopy zone (b) enlarged. For versatility, six measurement electrodes, and one forked electrode acting as signal output, was patterned onto the chip. The electrodes were platinum, with a thickness of 200 nm, a width of 20 µm and a space of 30 µm between adjacent electrodes. In these experiments the two electrodes closest to the fork on either side was used.](image)

We added an acoustic focusing zone to focus particles using standing wave ultrasound at 5 (vertically) and 2 (laterally) MHz respectively. 5, 7 and 10 µm polystyrene beads were suspended in 0.9 % NaCl MQ water. Blood was diluted 500 times in PBS. Flow was set to 5 µl/min. Differential impedance spectroscopy was performed at 3 MHz and 800 mV output. Acoustic forces was induced using piezoelectric transducers attached to the bottom of the chip. The transducers were actuated by signal generators. The raw data was analysed using the “findpeaks” function in Matlab and electric pulse amplitudes extracted together with differential (+)pulse to (-)pulse time values for each particle which can be used to evaluate flow speed between the two measuring electrode areas in the MIC. The polystyrene bead mix data from the MIC was compared with data using a Multisizer 3 Coulter counter in order to further evaluate MIC performance.
Results

Data shows that the MIC was able to prefocus cells and beads thus improving MIC performance. Peak amplitude histograms suggest good correlation between particle volume and peak amplitudes for the 5, 7 and 10 µm bead populations which was also confirmed in the Multisizer data, Figure 3. When prefocusing was activated distribution of pulse amplitudes for each particle size was better.

Conclusions

This opens up the path towards an integrated device with acoustophoretic pretreatment of a sample, for example raw milk, with particle sorting, alignment and subsequent cytometry on a single chip.

REFERENCES:
1. "Micromachined impedance spectroscopy flow cytometer for cell analysis and particle sizing", S. Gawad, Ph. Renaud et al., Lab Chip, 1, 76-82 (2001)
An extremely parallel acoustic flow cell for rapid cellular analysis

Steven W. Graves, Pearlson Prashanth Austin Suthanthiraraj, and Menake E. Piyasena

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Introduction
Flow cytometry has become an invaluable tool for a wide variety of cellular analyses ranging from determination of DNA content of cells to the detection of pathogens in blood and determination of multiple parameters of a single cell. Using the traditional hydrodynamic method of focusing, current flow cytometers achieve particle analysis rates as high as 50,000 events per second, while delivering sample at roughly 100 µl/minute. To increase throughput, attempts have been made by various researchers to achieve higher analysis rates by running four flow cytometer analysis heads in parallel. However, new application areas requiring detection of very rare events (e.g. circulating tumor cells) require dramatically higher throughput, both in terms of volumetric delivery and particle analysis rates. To develop flow cells that could support greatly increased flow and analysis rates, we have used acoustic standing waves to create flow cells that support many parallel streams of particles. In our recently published work, we have reported that as many as 37 parallel streams of particles can be achieved in an acoustic flow cell with a sample delivery rate of 1.6 mL/min [1]. However, these systems did not support the linear velocity required to support extreme throughput flow cytometry. Therefore, we have developed a massively parallel microfluidic flow cell using deep reactive ion etching techniques [2], which supports increased flow rates and numbers of focused streams, with the intended application of highly parallel flow cytometry.

Experiment
The acoustic focusing flow cell that we have used to demonstrate the generation of highly parallel streams is fabricated using silicon wafer and pyrex glass slide. It consists of a single sample input leading to 100 parallel channels that were photomasked onto silicon substrate using standard photolithography and etched using the deep reactive ion etching method. The pyrex glass slide is bonded to the silicon substrate by anodic bonding and silicone tubing of standard dimensions is attached at the inlet and outlet. Each channel is designed to generate three acoustic nodes that result in three well-defined streams of particles when the flow system is driven at its corresponding resonant frequency. A single PZT (lead zirconate titanate) transducer, attached to the base of the silicon substrate, serves to resonate the acoustic flow cell.
Fig. 1. A flow cell with one hundred 500 µm wide by 100 µm deep channels etched into a single silicon wafer. When driven at 4.48 MHz this is predicted to produce 300 flow streams.

Results

In our highly parallel acoustic device, we have been able to focus particles into 300 streams (3 streams per channel in 100 total streams). Furthermore, using an EMCCD camera for image analysis, we have demonstrated well-focused streams at flow rates as high as 15 mL/min (Fig. 2). We will also present focusing of cells using parallel flow channels, and approaches to perform parallel optical analysis.

Fig. 2. (a) Three flow streams containing focused 10 µm particles within a single channel a multi channel device. The dashed line indicates where stream width measurements were assessed from video images (b) Stream widths measured in pixels. When converted to microns this analysis indicated that streams were consistently well focused (~10 µm wide) and insensitive to increasing flow rates.

Conclusion

In this work, we have shown that it is possible to obtain hundreds of parallel streams of particles using acoustic forces at flow rates of tens of millilitres per minute. When paired with effective analysis techniques, this flow cell represents an important technological advance in parallel flow cytometry that will be an important component in extreme throughput cytometers for the analysis of extremely rare event applications such as the detection of circulating tumor cells and fetal cells in maternal blood.

References


Theoretical analysis of acoustic streaming and radiation forces in microchannel acoustophoresis

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Email: bruus@fysik.dtu.dk

Introduction
The motion of microparticles in lab-on-a-chip systems can be controlled by MHz-ultrasound-induced acoustophoresis. In water, acoustophoresis of particles with a diameter 2a larger than 2 µm is well-described by neglecting the viscosity of water and by letting the radiation force be the only acoustic force acting on the particle. For particles of radius a comparable to or smaller than a few times the viscous boundary-layer thickness δ ≈ 0.5 µm, viscous effects cannot be neglected. The radiation force becomes viscosity-dependent, and a significant Stokes drag force arises from acoustic streaming in the bulk generated by viscous stresses in the viscous boundary layer. In this talk we present a theoretical analysis of the acoustic streaming and the radiation force in microchannel acoustophoresis with emphasis on the dependence on viscosity.

Theory
Briefly, and to establish notation [1], for a given fluid in the absence of external forces and for isothermal conditions, the theory is based on a combination of the thermodynamic equation of state expressing pressure \( p \) in terms of density \( \rho \), the kinematic continuity equation for \( \rho \), and the dynamic Navier–Stokes equation for the velocity field \( v \). We consider a quiescent liquid, which before the presence of any acoustic wave has constant density \( \rho_0 \) and pressure \( p_0 \). Let an acoustic wave constitute tiny perturbations to first and second order (subscript 1 and 2, respectively) in density \( \rho \) and pressure \( p \), and velocity \( v \). The first-order equations are

\[
\begin{align}
\partial_t \rho_1 &= -\rho_0 \nabla \cdot v_1, \tag{1a} \\
\rho_0 \partial_t v_1 &= -c_0^2 \nabla \rho_1 + \eta \nabla^2 v_1 + \beta \eta \nabla (\nabla \cdot v_1), \tag{1b}
\end{align}
\]

where \( c_0 \) is the speed of sound of the fluid and \( p_1 = c_0^2 \rho_1 \). Both the radiation force and the acoustic streaming are time-averaged effects. Assuming harmonically varying first-order fields, e.g. \( \rho_1 = \rho_1 (r) e^{-i\omega t} \), all first-order fields vanish upon time averaging, and we must therefore study the time-averaged second-order equations,

\[
\begin{align}
\rho_0 \nabla \cdot \langle v_2 \rangle &= -\nabla \cdot \langle \rho_1 v_1 \rangle, \tag{2a} \\
-\nabla \langle p_2 \rangle + \eta \nabla^2 \langle v_2 \rangle + \beta \eta \nabla (\nabla \cdot \langle v_2 \rangle) &= \langle \rho_1 \partial_t v_1 \rangle + \rho_0 \langle v_1 \cdot \nabla v_1 \rangle. \tag{2b}
\end{align}
\]

Results
The time-average acoustic radiation force \( \mathbf{F}^\text{rad} \) on a single small spherical particle of radius \( a \), density \( \rho_p \), and compressibility \( \kappa_p \) in a viscous fluid is found by Settnes and Bruus [1] to be

\[
\mathbf{F}^\text{rad} = -\pi a^3 \left[ \frac{2\kappa_0}{3} \text{Re}[f_1^* p_1^* \nabla p_1] - \rho_0 \text{Re}[f_2^* v_1^* \cdot \nabla v_1] \right], \tag{3}
\]

where \( \kappa_0 = 1/(\rho_0 c_0^2) \) is the compressibility of the fluid, where the asterisk indicate complex conjugation, and where the pre-factors \( f_1 \) and \( f_2 \) are given by

\[
\begin{align}
f_1(\tilde{\kappa}) &= 1 - \tilde{\kappa}, \quad \text{and} \quad f_2(\tilde{\rho}, \tilde{\delta}) &= \frac{2[1 - \gamma(\tilde{\delta})](\tilde{\rho} - 1)}{2\tilde{\rho} + 1 - 3\gamma(\tilde{\delta})}, \tag{4a}
\end{align}
\]

with \( \tilde{\kappa} = \frac{\kappa_p}{\kappa_0}, \quad \tilde{\rho} = \frac{\rho_p}{\rho_0}, \quad \delta = \frac{\delta}{a}, \quad \text{and} \quad \gamma(\tilde{\delta}) = -\frac{3}{2} \left[ 1 + i(1 + \tilde{\delta}) \right] \tilde{\delta}. \tag{4b} \)
Figure 1: (a) - (b) Calculated viscosity-induced relative change $D$ (see the text) in the radiation force acting on polystyrene and pyrex microparticles, respectively, in saltwater as function of salinity. Adapted from Ref. [1]. (c) - (e) Numerical simulation of microparticle acoustophoresis for 10 s in the vertical cross-section of hard-walled rectangular microchannel of height 160 µm and width 380 µm filled with pure water of viscosity $\eta = 1$ mPa s and driven at the horizontal standing half-wave pressure resonance at frequency 1.97 MHz. Particle diameters are $2a = 0.5$ µm, 2 µm, and 5 µm, respectively. The dots indicate the end-position after 10 s of acoustophoresis, the lines the particle path, and the color the particle speed. Adapted from Ref. [2].

Note that only $f_2$ depends on viscosity. The relative change due to viscosity is measured by the factor $D = F_{\text{rad}}(\delta)/F_{\text{rad}}(0) - 1$, see Fig. 1(a)-(b).

The time-averaged Stokes drag force $F_{\text{drag}}$ on a spherical particle of radius $a$ moving with velocity $\mathbf{u}$ in fluid having the streaming velocity $\langle \mathbf{v}_2 \rangle$ is given by the usual expression

$$F_{\text{drag}} = 6\pi \eta a \langle \mathbf{v}_2 \rangle - \mathbf{u},$$

valid for particles sufficiently far from the channel walls.

Muller et al. [2] have made a numerical study of viscosity-dependent acoustophoresis as function of particle size, fully resolving the 0.5-µm-thin boundary layer, and using Eqs. (1) and (2) to calculate the velocity fields as well as Eqs. (3) and (5) to determine the acoustic forces. Part of the results are shown in Fig. 1(c)-(e), where the cross-over from streaming-dominated to radiation-dominated acoustophoresis is clearly seen: as the particle diameter changes from 0.5 µm to 5.0 µm the particle motion changes from a 2-by-2 vortex-mode to a mode with particle-focusing towards the vertical pressure nodal plane at the channel center.

References


Acoustic streaming in the transducer plane in resonant manipulation devices

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Introduction
Particle manipulation using radiation forces from ultrasonic standing waves has gained increased attention in recent years and is considered to be an ideal method for particle handling in microfluidic channels as it is non-invasive and requires no pre-treatment of the particles regardless of their optical or charge properties. During the process of particle manipulation, acoustic streaming is a well-known side effect that can disturb the manipulation process. In addition to classical Rayleigh streaming pattern, experimental work by Kuznetsova and Coakley [1] has described streaming patterns that occur in a plane parallel to the transducer, often over a multi-wavelength scale. The cause of these patterns has not previously been understood. Additionally, the same group [2] describe large multi-wavelength streaming patterns that do not break up in the manner described by Aktas and Farouk[3]. We present here a finite element method to numerically simulate the streaming patterns, utilising the boundary limiting velocity formulation described by Nyborg [4] and Lee and Wang [5] which predicts the effect of arbitrary boundary vibrations on the velocity of the local outer streaming flow.

Methods and results
The modelling was carried out in Comsol. First, a linear pressure acoustics interface was used to find the first-order resonant acoustic velocity field. Then, a computational fluid dynamics (CFD) model was used to obtain the fluid motion. The boundary-layer limiting velocities were derived as functions of the resonant field results from the linear acoustic model and applied to the CFD model as slip-velocity boundary conditions. The model geometry and simulation results are shown in Fig1.

(a)                                                                                   (b)
Fig1. (a) Numerical model of a glass capillary (0.4mm X 6mm) with PZT transducer; (b) Plan view of the 3D fluid motion
The experimental setup is shown in Fig2 (a). First, 10μm polystyrene beads were used to find the driving frequency, which was tuned until a strong focusing of the microbeads towards the centre of the channel was observed. Then, the ultrasound field was turned off and fresh microbeads were injected into the channel. When a homogeneous microbead distribution was observed, the flow was stopped and the ultrasound was turned back on. The transient movement of the microbeads was recorded by a CCD camera and the instantaneous streaming velocities were obtained from using matlab based PIV software. Fluid motion in in two adjacent fields in the capillary within the pressure nodal plane is shown in Fig2 (b).

The simulated streaming patterns compares well with the experimental visualisation. Methods to provide control of the streaming field within ultrasonic standing wave devices will be investigated in future work.

References
Acoustic streaming: Extension of Rayleigh’s analytical analysis to bounded microsystems

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Introduction
When applying Rayleigh’s classical theory [1] on boundary driven acoustic streaming to experimental microfluidic devices two problems arise: (i) Rayleigh assumes the channel height $h$ to be much smaller than the acoustic wavelength $\lambda$, whereas in typical microchannels both the height and the width $w$ are comparable to the wavelength. (ii) Rayleigh’s solution is calculated for an infinite parallel-plate channel, while the microchannel geometry is a bounded system, and the effect of the no-slip boundary condition on the side walls is significant. We present an extension of Rayleigh’s analytical theory on boundary driven acoustic streaming that allows for prediction of the acoustic streaming in experimental ultrasound standing-wave devices. Moreover, this solution presents an important reference case for benchmarking of numerical simulations. A sketch of the transverse cross section of a long straight microfluidic channel is shown in Fig. 1(a), with the pressure resonance indicated by the purple sinusoidal curves. The acoustic streaming is sketched by the 2-by-2 vortex pattern in Fig. 1(b). For resent reviews on acoustic streaming see [2, 3].

Figure 1: (a) End-view sketch of an acoustophoresis chip consisting of a long straight rectangular channel and a piezo transducer. The purple sinusoidal curves indicate the transverse standing pressure wave in the channel. (b) Sketch of the steady rotational flow rolls in the vertical cross-sectional plane of the channel. This boundary-driven acoustic streaming-velocity field is generated by the non-linear interactions of the standing acoustic wave in the $\mu$m-thin viscous acoustic boundary layer at the channel walls.

Theory
We consider an isothermal water-filled cavity bounded by acoustically hard walls. The Navier–Stokes equation, the continuity equation, and the thermodynamic equation of state are solved analytically using perturbation theory to second order. The first-order equations are solved in the frequency domain, while the second-order equations are solved for time-averaged quantities only. First, we extend Rayleigh’s analysis of the streaming-velocity field in an infinite parallel-plate channel to cope with the height being comparable to the acoustic wavelength. Subsequently, we consider a rectangular microchannel, which we analyze in terms of the analytical parallel-plate solution for the top-bottom and left-right parallel-plate systems, respectively, in an alternating and iterative manner using Fourier expansions.
Results
Using this iterative Fourier expansion approach we are able to make an analytical prediction of the acoustic streaming in rectangular microfluidic channels as illustrated for three different channel cross sections in Fig. 2. The transverse standing half-wave ultrasound resonance shown in Fig. 1(a) generate a steady streaming velocity inside the µm-thin viscous acoustic boundary layer at the top and bottom walls, resulting in a slip boundary condition for the bulk flow. Streaming is not generated at the left and right side walls, thus the no-slip boundary condition applies there.

![Image of analytical results](image)

**Figure 2:** Analytical result represented by vector plot (white arrows) of the time-averaged second-order streaming velocity and color plot of its normalized magnitude. The velocity field is shown for aspect ratios of (a) $h/w = 2$, (b) $h/w = 1$, and (c) $h/w = 1/5$. For the low aspect ratio channel, (c), the horizontally-elongated flow rolls fill the whole channel, while for the high aspect ratio channel, (a), the streaming velocity is only significant close to the top and bottom walls.

Conclusion
Our results allow for analytical prediction of the acoustic streaming in experimental ultrasound standing wave devices. Furthermore, this presents an important reference case for benchmarking of numerical models of the acoustic streaming in microfluidic channels.

References
Controlling the ratio of radiation and streaming forces in microparticle acoustophoresis

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Introduction
Ultrasound manipulation of sub-micron particles is challenging as the Stokes drag force from the acoustic streaming flow dominates over the acoustic radiation force for particle sizes below a critical value. In this work we present measurements of microparticle acoustophoresis using an automated micro-PIV system (Augustsson et al. Lab Chip 11, 4152, 2011). We measure the particle velocities in the horizontal ultrasound symmetry plane at the mid height of the microchannel, \( z = 0 \), where the acoustic streaming and the radiation force are in-plane and parallel, and both attain a sinusoidal transverse profile. Fig. 1 shows a numerical simulation of the vertical microchannel cross section with (a) the transverse standing ultrasound pressure wave, (b) the motion of small microparticles dominated by the Stokes drag force from the boundary-induced streaming, and (c) the motion of larger microparticles dominated by the radiation force.

Figure 1: Numerical simulation of the acoustophoresis in the vertical microchannel cross section of width 380 \( \mu \text{m} \) and height 160 \( \mu \text{m} \): (a) Transverse standing ultrasound pressure wave \( p_1 \) from \( -p_a \) (dark blue) to \( p_a \) (dark red). (b) Particle trajectories of small particles subject to the Stokes drag force from the boundary-induced streaming. (c) Particle trajectories of large particles moving due to the acoustic radiation force. At the plane of measurement (magenta line), the streaming flow and radiation force are parallel and both attain a sinusoidal profile.

Theory
We study a standing transverse pressure \( p_1 = p_a \sin(ky) \) of amplitude \( p_a \) and wavenumber \( k \). At the mid height of the channel we obtain the acoustic streaming \( u_{str} \) (Rayleigh, Phil Trans R Soc London 175, 1, 1884) and the acoustic radiation force \( F_{rad} \) on a suspended particle of diameter \( 2a \) (Yosioka and Kawasima, Acustica 5, 167, 1955)

\[
\begin{align*}
  u_{str} &= s \frac{4E_{ac}}{\rho c} \sin(2ky), \\
  F_{rad} &= 4\pi a^2 (ka) E_{ac} \Phi \sin(2ky),
\end{align*}
\]

where we have introduced the acoustic energy density \( E_{ac} \), density \( \rho \) and sound speed \( c \) of the suspending medium, the particle/medium acoustic contrast factor \( \Phi \), and the geometry factor \( s \) being \( 3/16 \) in Rayleigh’s prediction for a parallel-plate channel, where the wavelength is much larger than the channel height. A particle suspended in a medium of viscosity \( \eta \) is subject to the Stokes drag from the streaming and the radiation force resulting in the particle velocity \( u_p = F_{rad}/(6\pi \eta a) + u_{str} = u_a \sin(2ky) \), and hence from Eq. (1) \( u_a \) becomes

\[
\frac{u_a}{u_0} = \left( \frac{a}{a_0} \right)^2 + s, \quad u_0 = \frac{4E_{ac}}{\rho c}, \quad a_0 = \sqrt{\frac{6\eta}{\rho \Phi \omega}}.
\]

1
where $\omega$ is the angular frequency of actuation. Moreover, the ratio of the streaming- and radiation-induced particle velocities is $u_{\text{str}}/u_{\text{rad}} = 6s \eta/(\rho \Phi \omega a^2)$.

**Experiments**

We examined a suspension of polystyrene microparticles with diameter $2a$ ranging from 0.6 µm to 10 µm suspended in either pure Milli-Q water or a 3:1 mixture of water and glycerol at constant temperature of 25 °C. The pure water solution was driven either at frequency $f = 1.940$ MHz or $f = 3.900$ MHz, while the glycerol solution was driven at $f = 2.027$ MHz.

**Results**

In Fig. 2 we show the result of a single micro-PIV measurement; (a) shows the axial velocity (left) and transverse velocity (right). Clearly, the system sustains a transverse half-wave resonance yielding a full sinusoidal velocity profile as predicted, see (b). In Fig. 3(a) we plot $u_a/u^*$ as function of the particle diameter $2a$ and where $u^*$ is the velocity of the largest 10-µm particle. As predicted, the velocity has the $a^2$-dependence and the approximated ratio $u_{0.6\ \mu m}/u_{10\ \mu m}$ of streaming- and radiation-induced velocity scales linearly with the material factor $\eta/(\rho \Phi)$ (largest for glycerol) and inversely with frequency $\omega$. In Fig. 3(b) we use table values for the material parameters to collapse all data as predicted by Eq. (2).

**Conclusions**

We have determined the ratio of streaming- and radiation-induced particle velocities and validated theoretical predictions that the ratio scales linearly with the particle size squared $a^2$, linearly with the material factor $\eta/(\rho \Phi)$, and inversely with the actuation frequency $\omega$. Finally, by proper rescaling, all measured velocities collapse on a universal curve. We have shown control of the force ratio, which paves the way for future use of streaming- and radiation-induced acoustophoresis for manipulation of sub-micron particles.
Open Chip for Biomolecule Sample Handling
SAW-MALDI MS

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Introduction
An open microfluidic device for handling of small volumes (< 1 µL), has been interfaced to MALDI MS[1]. Surface Acoustic Wave (SAW) atomizer consist of a piezoelectric material with two single-phase unidirectional transducers (SPUDTs) electrodes driven at 30 MH[2-4]. High energy SAW generates in 15 seconds small aerosols (2-10 µm diameter) from a deposited droplet. No molecular damage has been shown via SDS-PAGE[3]. SAW propagate and atomize the membrane-bound liquid sample. The membrane aids sample-pre-purification, by entraping impurities within the membrane fibers. SAWs self-pumping effect, generates direct sample transportation to the MALDI plate and avoids the risk of clogging; often a problem of closed microfluidic devices. SAW atomizer is here used primarily in rapid metabolic screening of islet of Langerhans-endocrine cell clusters involved in the pathogenesis of T2D. However due to the membrane format which favors non-invasive sampling and the instant, non-chemical extraction, the atomizer has been used in rapid body fluid protein/peptide profiling (saliva, gingival fluid, tear film). Minimal sample requirements, fast and easy operation, makes SAW an attractive tool in future diagnostics. Single/few cell analysis, in the context of T2D molecular studies, has also been performed in “wall-less test tube” via the acoustic levitation technique[5, 6]. With such approach, it is possible to handle small volumes without any adsorption to walls or related contamination. Flow-through droplet dispensers are used to deliver stimulators/inhibitors to the cells within the levitated droplet. Protein/peptide data from single β-cell and single islet and has been achieved by the combination of the airborne system and MALDI MS.

Experiment
Freshly prepared islet(s) were pipetted on a piece of membrane, placed on the atomizer and supplemented with buffer (30 mM glucose) bound to the membrane. Acetylcholine (100 µM) was then applied and allowed to stimulate the resting islet for 5 minutes. Following the islet stimulation, SAWs were generated. The resulting aerosol, rich in islet’s cell releasates, was collected on the MALDI plate via a pinhole (Figure 1). The alpha-cyano-4-hydroxycinnamic acid matrix (CHCA), applied on the same membrane, was SAW-extracted and deposited on the plate in a similar fashion. The described procedure developed by us, which was essential in qualitative spectra acquisition, is also refereed as SAW-deposition. After each experiment, the piezoelectric substrate was cleaned with acetone, isopropanol and rinsed with milliQ water as described by Ho[4]. Single β-cell within a levitated droplet (500 pL, 5mM glucose Hepes

Figure 1. (A) The SAW atomizer’s working principle and experimental setup. The atomizer consists of a piezoelectric material with two SPUDT electrodes which generate unidirectional Rayleigh-wave SAW that propagate towards and underneath the sample. High energy SAWs drives liquid atomization even when the liquid is previously sampled on a membrane. The resulting aerosols are collected on a MALDI plate via a pinhole for MS analysis.
buffer) were stimulated for 5 min with 5\(\mu\)M acetylcholine that was delivered to the droplet via a piezoelectric dispenser[5]. All samples were collected to a MALDI plate prepared with a crystal layer of CHCA and allowed to crystallise.

**Results**

Fast MALDI MS spectra generated from stimulated, intact islets of healthy mice are shown in Figure 2A. As expected, high glucose concentrations (30 mM glucose) in combination with acetylcholine (100 \(\mu\)M) has triggered \(\beta\)-cells to release insulin and other co-secreted molecules. A weak peak corresponding to the suppressed glucagon release from \(\alpha\)-cells is also indicated in the spectra. The acquisition of qualitative spectra, characterized by high S/N values, reproducibility and a LOD of less than 100 attomole, are attributed to the novel way of SAW based sample-matrix deposition procedure—a essential step of MALDI analysis. Rapid MALDI MS peptide profiling of biofluid (saliva, gingival fluid, tear film) sampled and preserved on a membrane has been also achieved. Figure 2B shows the tear film acquired spectra, sampled on a membrane and air-dried prior analysis. MALDI MS spectra acquired from a levitated single \(\beta\)-cell (Fig. 2C), induced (5 mM glucose, 5\(\mu\)M acetylcholine) for 6 minutes are characterised by strong signals of insulin, C-peptide and amylin.

**Conclusion**

The combination of two airborne analytical systems, the acoustic levitation and SAW-atomizer, with MALDI MS has been achieved. The acoustic levitation shows an outstanding sensitivity (20-80 attomole) in protein analysis released from single \(\beta\)-cell. However, single islet analysis has also been done using the SAW atomizer—a fast and easy sample handling tool for MALDI MS. Acetylcholine-stimulated islet’s releasates, sampled on a membrane, were extracted via SAW and detected with MALDI MS. Since the sample here is minimally chemically pretreated the risk of MALDI artefacts is considerably reduced. The reproducibility, and sensitivity (less than 100 attomole) of SAW-MALDI is mainly attributed the newly developed SAW-based sample-matrix deposition method. Besides performing an instant non-chemical, membrane purification/extraction of the sample, the chip reduces the use of vials, containers and related analysis cost. The membrane format of the SAWs operation allow for non-invasive sampling of small volumes biofluid (less than 1 \(\mu\)L) and related MS-based diagnostication.

**References**

Sessile droplet resonances and low power SAW actuation

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Introduction
Actuators based on surface acoustic waves are versatile tools to achieve atomization, jetting, oscillations, or inner mixing of droplets [1]. Basically, acoustic waves are generated at the surface of a piezoelectric substrate by a transducer consisting of interdigitated fingers. The acoustic energy is then transmitted to a drop lying on the substrate; its motion is then induced by nonlinear acoustical effects (acoustic streaming, acoustic radiation pressure). However, an increase in the temperature occurs inside the drop due to the dissipation of acoustic energy. This could be harmful for the manipulation of biofluids. In this presentation, we will show that the amount of energy required to move or deform droplets can be drastically reduced by exciting them at their inertia-capillary eigen frequencies.

Experiment

Fig. 1. a) Scheme of the experimental setup. b) Droplet response to the SAW: combination of dipolar oscillations and translational motion.

Surface Acoustic Waves (SAW) are generated at the surface of a 1.05 mm-thick piezoelectric substrate (X-cut niobate lithium LiNbO3) by a transducer consisting of interdigitated fingers. The width of the fingers and their distance are both equal to 43.75 µm, leading to a characteristic frequency of 19.5 MHz, which is used as the carrier frequency fc. A periodic sinusoidal voltage is applied at this frequency with a high frequency generator (IFR 2023A) and amplified with a homemade amplifier. This carrier signal is modulated by a square wave switching between 1 and 0, at frequency fm << fc. The amplitude d of the SAW is measured with a Mach-Zender laser interferometer (BMI-SH130). The surface of the substrate is treated with hydrophobic coating.
(monolayer of OTS) leading to advancing and receding contact angles of $\theta_a = 108^\circ$ and $\theta_r = 99^\circ$, respectively, measured with a Kruss DSA100 goniometer. A 7.5 µL droplet of water is then placed on the substrate. The droplet dynamics is observed via a high speed camera (Photron SA3) and recorded at 2000 frames per second. To avoid pollution of the surface by impurities and to obtain reproducible results, all the experiments have been carried out in our laboratory class 1000 clean room.

**Results**

Droplets exhibit dipolar oscillation modes whose eigen frequency is fixed by the competition between inertia and surface tension [2-3]. When the acoustic signal is modulated around these characteristic frequencies, some resonance effects are evidenced (see Fig. 2), which lead to larger drop oscillations but also larger translation speed. The droplet response is either superharmonic (at twice the modulation frequency), harmonic or subharmonic (at half the frequency of excitation). It can be explained through weakly nonlinear analysis of droplet oscillations modes.

![Fig. 2](image.png)

**Fig. 2.** Amplitude of vertical oscillations of the drop $\Delta h$ divided by the initial height of the droplet $h_0$ as a function of the modulation frequency $f_m$ for different amplitudes $d$ of the surface acoustic wave. In the green, blue, and red region, the droplet response is, respectively, superharmonic, harmonic, and subharmonic (compared to the frequency of modulation).

**Conclusion**

In this presentation, we will evidence droplet harmonic, subharmonic and superharmonic resonances and show how the droplet oscillations promote their translational motion. This can be of primary interest to reduce the amount of energy required to move and stretch droplets for lab-on-a-chip applications.

**References**

Cell Deformation with Acoustic Radiation Forces

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There has been recent interest in the optical tweezing community in the possibility of stretching biological cells using optical radiation forces [1]. By observing the cells as they are compressed it is possible to assess the mechanical compliance of the cells, with potential applications in, for example, identifying cancer cells [2] and parasite infections such as malaria. We demonstrate here a similar effect that uses acoustic radiation forces to compress levitated cells. The acoustic deformation can potentially be applied to many (e.g. thousands) of cells simultaneously, leading to higher throughput diagnostic devices.

The forces on a particle that cause it to deform have previously been reported and modelled in the context of levitated liquid droplets by Marston [3], where surface tension opposes the deformation. Jackson et al. [4] extended this work to be valid for larger values of $ka$ and, Tian et al. [5] gave results that were valid for deformations up to an aspect ratio of 2.

In our system, osmotically swollen red blood cells (RBCs) are used to demonstrate the principle as they are particularly compliant due to the absence of a cytoskeleton. A resonance is formed in a square capillary of inner dimension, 100µm. Excited by a transducer at the half-wave resonance of 7.9 MHz, cells are both levitated and focussed laterally into a single line down the centre of the capillary. Observation through the side of the capillary allows bright-field or phase-contrast microscopy to be used to accurately assess cell shape. The compression occurs perpendicular to the direction of observation, allowing the deformed shape to be seen in profile. Initial results can be seen in Figure 2, which shows a RBC at low and high levels of acoustic forces.

We construct a finite element model of the deforming cell in COMSOL multiphysics, representing the RBC membrane with shell elements. Using equations for the acoustic radiation stress tensor [6] as a function of first order acoustic fields, the results from a linear acoustic model are used to calculate the radiation stress on the membrane. The model is run as time dependent, and allowed to reach equilibrium to encourage convergence. At each time step the mesh position is updated and the corresponding acoustic forces re-calculated in order to account for the non-linearities of larger deformations.
Figure 2: A Red Blood Cell is deformed. (a) Aligned cell in capillary (b) 10 Vpp excitation (c) 50 Vpp excitation. The corresponding pressure amplitudes are omitted until more careful investigation has verified our initial estimates. Images in bright-field illumination.


Finding cancer cells in blood using ultrasound

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Introduction
According to the world health organization (WHO) the number of cancer related deaths will increase from 8 million in 2008 to 13 million worldwide in 2030. Most cancer related deaths are caused by lung-, breast-, colon-, stomach- and prostate cancer. Cancers cause metastases at distant sites through circulating tumor cells (CTC), which escape the tumor via the blood stream and settles primarily in the lungs or the liver. Isolation of CTCs from patient blood has gained a lot of interest in the recent past since it holds promise for early discovery of metastatic disease.

Several strategies have been proposed such as physical [1], and affinity based approaches involving microbeads [2] or micro-posts [3]. The large variations not only in morphology but also in antigen expression among CTCs, call for novel approaches to enhance CTC enrichment. We propose a flow-through separation approach, which utilize acoustophoretic forces to extract CTCs from blood.

Method
An acoustophoretic chip separates cells based on size, density and compressibility. The layout was first demonstrated for separation of blood components by Petersson et al. [4]. Samples of red blood cell-lysed blood and spiked prostate cancer cells were separated in the device (Fig. 1). The cells are first hydrodynamically positioned near the walls at the inlet of a separation channel. While flowing through the channel, the cells are pushed towards the center of the flow and are separated according to the acoustophoretic mobility of the cells. At a trifurcation outlet, cancer cells (high mobility) are collected in a central branch while blood cells (low mobility) are collected in the side branches.

Experiments
Samples of prostate cancer cell line DU145 (2.5 x 10⁵ mL⁻¹) spiked in red blood cell (RBC) lysed whole blood (diluted 1:10), were fixated and processed in the acoustophoresis chip at different flow rates in order to map the separation efficiency.

Due to large variations in morphology among CTCs occurring in clinical samples, separation efficiencies for three different prostate cancer cell lines were investigated. Prostate cancer cell lines DU145, LNCaP and PC3 (2.5 x 10⁵ mL⁻¹) where spiked in RBC lysed whole blood and processed in the chip.

To investigate any potential cell damage caused by the ultrasound, live cells from all three cell lines were run through the separation channel at low flow rate and high ultrasound intensity. After one passage through the device the percentage of viable cells was estimated by Tryptan blue staining followed by counting of viable respectively dead cells in a Bürker chamber.
Results
Figure 2 shows that acoustophoretic separation of DU145 cells and WBCs is feasible. The cancer cell recovery in the system can be tuned to above 90% while suppressing the WBCs to below 10%. Cancer cells from three different cell lines could be successfully separated from WBCs in the system. Cells maintain viability and biological functions after passage through the device (Data not shown).

Conclusion
We provide a proof of principle by showing that tumor cell enrichment by acoustophoresis may have potential for use in clinical diagnostics of CTCs in cancer patients.

References
Directing suspended samples by USW in vibrational spectroscopy macrosensors

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Introduction
Within the past five years we have pursued the enhancement of vibrational spectroscopy methods by ultrasonic standing waves. This research was driven mainly by the need of reliable sensors in the bioprocess environment. Biotechnology is used intensively as manufacturing route for all sorts of compounds (medical, industrial, etc.) and vibrational spectroscopy is increasingly popular in process analytical chemistry because of its ability to directly provide molecular specific (bio-)chemical information about a given sample. Beside feasibility of the exploitation of an USW for Raman spectroscopy [1,2] we concentrated on the enhancement of ATR (Attenuated Total Reflection) spectroscopy.

Experiments
The ATR spectroscopy is a widely used method for mid-infrared absorption spectroscopy especially in connection with highly absorbing samples like e.g. aqueous solutions. Only a thin film of some micrometers in the proximity of the ATR sensitive element is spectroscopically analysed, beyond this evanescent field the instrument is “blind”.

USW exploited to decrease biofilm formation
On one hand this poses sensitivity problems, when this light skin, where the actual measurement takes place, is blocked by the formation of biofilms. This was encountered for yeast suspensions on a horizontal ATR sensing element like used in a flow cell exploiting the stopped flow principle, see Fig.1 (a). It was shown [3], that the application of an USW during the rinse could decrease the build-up of a biofilm substantially as shown in Fig.1. (b) and (c). A comparison with various chemical means delivered, that the application of an USW performs well while lacking the need of aggressive chemicals or increased rinse times e.g. to empty the chamber of certain solvents [4].

![Fig. 1.](image_url)

Fig. 1. (a) Sketch of flow cell comprising the ATR element at the bottom and the PZT-sandwich transducer at the top. (b) Biofilm formation on the ATR during the protocol by yeast cells inefficiently rinsed between measurements. (c) Decrease of biofilm, when an USW was used to lift the residuals on the ATR during rinsing.
USW enables specific in-line ATR spectroscopy

However, on the other hand the small volume of the sensitive region opens the possibility to exploit the ability to manipulate the whereabouts of suspended particles by radiation forces. The USW is used is used to push the particles towards the optical sensor like in Fig.2 (a) resulting in the possibility to measure their infrared spectrum while in suspension.

Experiments with sample suspensions of polystyrene resin beads (100 μm) in methanol and PTFE particles (0.25 μm) in tetrahydrofuran showed, that absorption signals as well as the a decreased distance assessed by photographic images of the ATR surface coincided with the application of a certain “pushing” frequency, while at a different “retracting” frequency a decreased absorption and a gap between particle agglomerates and sensing element were detected [5]. This suggested the successful population and depopulation of the evanescent field with particles.

As mentioned the technology is of special interest in the bioprocess environment, the mid-infrared spectroscopy is hoped to be capable of delivering information about the physiological state of the cells. A further study was therefore conducted recently [6] with yeast suspensions of different concentrations in an experimental set-up – a gently stirred beaker. It was possible to control the biomass contained in the evanescent field of an in-line ATR probe by switching the frequency between the two values identified as “pushing” and “retracting” beforehand, see Fig.2 (b).

**Fig. 2.** (a) Measurement principle of USW enhanced ATR spectroscopy. Suspended particles (cells) are pushed onto the surface of the ATR element. Spectra are therefore dominated by the chemical composition of the agglomerates (biomass). (b) Carbohydrate signal over time while the ultrasonic frequency was alternating set to the “pushing” and the “retracting” value.

**Conclusion**

The presented data about exploiting an USW for the enhancement of vibrational spectroscopy techniques are promising. Especially the envisaged measurement of the chemical composition of the biomass and the supernatant independently would be a new tool in bioprocess technology. Such data assessed in-line would enable one to gain better control over a fermentation.

**References**


Sound wave reflections

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Our aim
To design a system where the dominant losses are due to moving particles to the pressure nodes in the fluid.

Decoupling vibrations to the reflector
How a resonator is held is often critical to its success as a resonator. For example a bell is held at its apex which is a velocity node, a glockenspiel plate is supported about a 1/5 of its length from the ends, also velocity nodes. If these velocity nodes are fixed without any degrees of freedom energy is rapidly transferred to the support structure and the resonance dies out. Resonant chambers used for particle manipulation are driven by external vibrations, this constant energy input makes these systems more forgiving for the experimenter however for manipulation of the smaller particle diameters or when driving larger chambers a strong resonance is still needed. In many systems the resonant chamber is supported by holding a vibrating part of the chamber, here instead the reflector is held securely, correctly clamping the reflector improves the resonance and since many models expect the reflector to be secure and not coupled to the driving vibrations the agreement with models is improved.

In the construction used here (figure 1) the aim is for all losses to occur in the fluid and not the structural parts. Vibrations pass from the PZT into a vibrating section and then on to the liquid. The wall beyond the liquid is held firmly and has only minimal structural connections to the vibrating plate. Particles moved rapidly when the reflector was held tightly and remained almost stationary when the reflector was free to move with the rest of the system. Ideal reflectors should be a quarter-wavelength thick, we have compared reflectors with arbitrary thickness and quarter-wave thickness.

Figure 1 Diagrams of the resonant chambers with fixed reflectors A, an arbitrary reflector thickness. B a quarter-wavelength thickness reflector.
**Structural Continuity**
Streaming occurs in chambers for many reasons, all related in some way to variations in field intensity. Some sources of streaming cannot be eliminated since vibrations never have completely uniform intensity, here we report on some vigorous streaming which can be eliminated but often goes unnoticed. The streaming occurs between two surfaces in close proximity (~10 µm) much closer than the 1500 µm wavelength used. This is something that is found when a chamber wall is not in full contact with the reflector or another wall.

The system used is, a half-wavelength scale duct connected to a much smaller duct. The streaming dissipates the energy in the standing wave as the velocity node at the wall is lost to the moving fluid. This type of streaming is usually only discovered if the gap is large enough for the type of particles used to enter. The absence of standing waves producing particle movement in the main duct is often the first indication that this thin layer streaming is present.

![Streaming in very thin layer section](image)

*Figure 2 Streaming occurs in layers much thinner than one-wavelength next to resonant channel.*

**The conclusions to achieve our aim**
1/ The reflector should not be in contact with any vibrating part other than the fluid.
2/ There should be no thin fluid filled gaps between the reflector and other vibrating parts.

A system which largely fulfils these two criteria will be presented.
Positioning performance in a ultrasound actuated concave-shaped multiwell microplate by a rotational-symmetric ring transducer

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Introduction
We have previously described a gentle method for measuring individual cell-cell interactions based on ultrasonic trapping of cells in a multiwell microplate combined with ultrasonic wedge transducers [1]. This device has been used for imaging of the immune synapse formed between natural killer (NK) cells and target cancer cells [2]. Although the wedge transducers have proven to be useful in practical applications they have a complicated mechanical behavior which is difficult to predict. In the present paper, we demonstrate a novel rotational-symmetric ultrasound ring transducer. We quantify the positioning performance of few-particle clusters at different schemes of ultrasonic actuation (including different single frequencies as well as frequency sweeps) in a concave-shaped multiwell microplate, and we use this information with the aim to optimize high-resolution 3D confocal microscopy screening of cellular clusters trapped in the microplate.

Experiment
A schematic view of the device is shown in Fig. 1a. The device consists of the concave-shaped multiwell microplate being fixated to the ring-shaped PZT piezo crystal by the variable spring-loaded holder. To visualize the acoustic trapping, 10 µm-sized green-fluorescent polystyrene particles were used in a mixture of Milli-Q water and Tween-20 yielding a concentration of 9.1 × 10⁴ beads per mL. An inverted bright-field microscope was used in epi-fluorescent mode together with a video camera. The videos were processed and analyzed with Matlab image processing toolbox.

Results
A microscope image of the trapping result for one single frequency (SF) actuation compared to frequency modulation (FM) actuation is shown in Fig. 1b. A more detailed analysis of the shape and position of few-particle clusters trapped at different single frequencies, as well as at FM actuation, is shown in Fig. 2. On average, FM actuation decreases the field of view (FOV) of the microscope to merely 13.4% ± 4.8% of the corresponding FOVs when using SF actuation. Furthermore, the average distance from center of the well to the center of the clusters decreases from 57 µm ± 36 µm to as little as 14 µm ± 10 µm, approx. a factor of 4, when using FM actuation compared to SF actuation.

Conclusion
Our results show that when implementing FM actuation both the accuracy and precision of the acoustic trapping of few-particle clusters increases. Thus, the scanning time of 3D confocal microscopy during high-resolution imaging of the immune synapse (cf. Ref. 2) can be decreased one order of magnitude with FM actuation compared to SF actuation.
References:

Fig. 1. (a) Schematic view of the device (ultrasound transducer and multiwell microplate). (1) Springs. (2) Aluminum clip. (3) Polymethyl methacrylate (PMMA) spacer. (4) Cover glass. (5) Micro coaxial (MCX) connector (6) PDMS frame. (7) Multiwell microplate. (8) Ring-shaped PZT piezo crystal. (9) Aluminum baseplate. (b) Trapping result with 10 µm-sized tracer particles in all 100 wells for FM (2.30 MHz ± 100 kHz @ 1 kHz, 50 Vpp, yellow) and one SF (2.23 MHz, 50 Vpp, red) actuation.

Fig. 2. (a) Distances from the center of the well to the center of the clusters for SF (f1, ..., f9 = 2.20, 2.23, 2.26, 2.29, 2.30, 2.33, 2.36, 2.39, and 2.40 MHz, 50 Vpp, red) and FM actuation (2.30 MHz ± 100 kHz @ 1 kHz, 50 Vpp, yellow). Here vertical error bars (red) and horizontal dashed lines (yellow) indicate the standard deviations, and the solid line (yellow) and filled red circles mark the average distances. (b-j) Positioning accuracy as a function of field of view (FOV) for SF actuation (f1, ..., f9, red clusters) and for FM actuation (2.30 MHz ± 100 kHz @ 1 kHz, 50 Vpp, yellow clusters). For each frequency, f1 through f9, the result from 10 selected wells are superimposed into a single well. The two squares for each frequency indicate the minimum well-centered FOV needed to cover all clusters under FM actuation (green) and SF actuation (magenta). Under each well the FOV ratio for FM and SF actuation is given in percent.
Micromotors like metallic nano or microrods can be propelled by external electric or magnetic fields, in this case objects move according to their mobility and the specific conditions of the applied field, or propelled by local conversion of energy, in this case objects move autonomously forming a called “active fluid”. Autonomous micromotors show a biomimetic behavior [1, 2] such as chemotaxis, swarming, and seem interesting for drug delivery applications. Nevertheless, motors with solutions of high ionic strength and the use of toxic fuels have limited their applications in biologically relevant media.

In this study, we demonstrate that ultrasonic standing waves in the MHz frequency range can levitate, propel, rotate, align, and assemble metallic microrods (2 µm long and <330 nm diameter) in water as well as in solutions of high ionic strength (Figure 1). Fast axial motion of metallic microrods at ~200 µm/s was observed at the resonant frequency using continuous or pulsed ultrasound. Segmented metal rods (AuRu or AuPt) were propelled unidirectionally with one end (Ru or Pt, respectively) consistently forward. A self-acoustophoresis mechanism based on the shape asymmetry of the metallic rods could be evoked for explaining this axial propulsion. We shall describe the structures formed by different parameters such as frequency, amplitude, concentration and microrod nature. Microrod propulsion is not directly related to the acoustic streaming phenomenon, experiments using mixtures of microrods with nanometric-sized particles nicely illustrate that difference.

Figure 1: Electron micrographs of Au (a) and AuRu (b) rods used in the ultrasonic propulsion experiments. (c) Typical ring pattern formed by AuRu rods. (d) Typical motion types of metal micro-rods in a 3.776 MHz acoustic field.

Particle manipulation with a looped waveguide resonator

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Introduction
Most ultrasonic particle manipulation devices described in the literature utilize reflections at fluid-structure interfaces which create a standing wave. At a given resonance frequency, the nodal planes are fixed since their locations are governed by the geometry of the device. This reduces the suitability of the method for applications that require particle transport over long distances or particle positioning at arbitrary locations. In order to overcome the described limitations, a number of approaches has been investigated in the literature [1]. One way is to use circumferential resonances within a loop-shaped fluid waveguide. In this abstract, we describe two device designs which are suitable to create and move circumferential resonance patterns within a circular fluid channel. Experimental results confirm the analytical model and illustrate potential applications.

Device Design
A fluid waveguide resonator can be built with materials that provide a high acoustic impedance mismatch between the fluid and the structural part of an acoustic manipulation device. Particularly interesting are the circumferential resonances in a loop-shaped fluid waveguide. Here, the pressure nodal positions are not constrained by boundary conditions. Consequently, standing waves can be shifted arbitrarily along the channel axis. In this case, the position-defining feature is the energy supply to the resonance mode and thus the excitation. In a typical setup, the transducer is attached to a structural part of the device and excites fluid resonances indirectly over structural vibrations of the device body. This emphasizes the importance of two factors which both affect the fluid structure interaction: the geometric design of the excitation and the material choice of the device body. Owing to the high impedance difference between the device body and the fluid, high pressure amplitudes in the fluid can only be obtained if the structural stiffness of the device body is decreased at the excitation position. This can be achieved by either locally using a more compliant material or by geometrically weakening the structure between the transducer and the fluid channel. An alternative way to excite the fluid resonances exists if the fluid channel is not closed by a cover lid. Transducers can be arranged such that they touch the otherwise free fluid surface. In this way, the fluid resonances can be excited directly with minimal structural vibrations of the device body.

Method
There are different ways to move the resonance pattern in a controlled fashion. The obvious one is to use a transducer that can be moved along the fluid channel. The resonance field and the particles follow the motion of the transducer [2]. Another option is to have a setup with two fixed transducers. If they are positioned properly, it is possible to excite two shifted standing waves. Superposition of the latter results in a standing wave whose position depends on the amplitude ratio of the transducers. The full one-dimensional control over the position and the velocity of the resonance pattern can be achieved with an amplitude modulation technique [1].
Experiments

Particle positioning and transport is shown experimentally using two different device designs, both of which have a device body made of tungsten-copper. Steel works as well but tungsten-copper with its higher acoustic impedance leads to an improved decoupling between the fluid resonance and undesired structural vibrations. Figure 1 shows two device designs which both have a circular fluid channel inside a disc-shaped device body.

Driving only the movable transducer of the device shown in Fig.1(a), it is possible to move the resonance pattern according to the manual motion of the transducer. Independent of the transducer position, the fluid channel stays in full resonance, leading to high pressure amplitudes and radiation forces. The position of the particle lines can be adjusted very accurately, which recommends this setup for one-dimensional tweezeing applications. Using both transducers of the experimental setups shown in Fig.1(a) and Fig.1(b) allows for the alternative way to move the resonance pattern via amplitude modulation. This working principle has the advantage that no moving parts are necessary and it is better suited for miniaturization and automatization. The motion of the resonance pattern agrees with the analytical predictions. This means the particle lines can be positioned at any location along the channel whereas the velocity and the direction of the motion can be controlled as well. Figure 2 shows the position of the particle lines during two amplitude modulation cycles. The resonance pattern travels over one wavelength ($\lambda = 3.5$ mm) during each modulation period ($T = 0.78$ s).

Fig.1: These two manipulation devices have a loop-shaped fluid waveguide with an open upper surface, a width of 1 mm, a depth of 1.5 mm and a length of 60 mm. (a) Two piezoelectric transducers touch the fluid surface but not the device body. One of them is at a fixed position, the other one can be moved manually along the circular fluid channel. (b) Two transducers are glued into notches at the sides of the device body. Between the piezoelectric element and the fluid channel there is a thin wall with a thickness of 0.3 mm.

Fig.2: Due to the one-dimensional resonance field at a frequency of 510 kHz, the 5-60 $\mu$m glass particles form thin lines at the pressure nodes. The pictures show a partial view of the fluid channel at different instances during two amplitude modulation cycles.

There are still a number of unresolved problems concerning the stability of the experimental setup. Most importantly, due to evaporation at the free water surface, the resonance frequencies shift rapidly. To date, this limits the operation time to a couple seconds but work is underway that aims to increase the stability and the reproducibility.

Particle separation in microfluidic channel using a switching ultrasound standing wave

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Introduction
Micro-sized particles can be separated based on their mechanical properties using an ultrasonic standing wave in microfluidic channels [1-3]. Typically, particles with opposite contrast factors are separated by the acoustic radiation force, which pushes particles with positive contrast factor to the pressure node and those with negative contrast factors to pressure antinode [2, 3]. The acoustic contrast factor depends on the differences in density and compressibility of the particles with respect to the surrounding fluid medium. However, for biological applications, most species of cells, such as isotonic erythrocytes and bacteria, exhibit positive contrast factors when suspended in aqueous solution. The separation process would have to make use of the relative strength of the radiation force acting on the different cells or particles. In this paper, we present a new methodology that makes use of an ultrasonic standing wave, which switches between the first and third mode, to effectively separate two species of particles with the positive contrast factors. Similar ideas had been reported in the literature [4-7], but using different modes of the ultrasound field and implementation in the fluid channel.

Methodology
The operating principle of our design is illustrated in Figure 1. The particles are first focused near the lower nodal line [A-A] of the third mode. When the standing wave is switched to the first mode, the particles start to move towards the center node [C-C]. Larger particles experiencing a larger acoustic force move faster towards the center node. The third mode is switched back on before the slow-moving smaller particles cross the maximum anti-node barrier [B-B] midway between the two nodal lines. The larger particles would have crossed the barrier [B-B] when the third mode is switched back on. Hence, the larger particles stay on nodal line [C-C], which remains as a nodal line for both the first and third modes. After several cycles of switching between the first and third modes of the standing wave, the large and small microspheres are effectively separated on two sets of nodal lines. The smaller particles accumulate on the lower nodal line [A-A] and larger particles on the center nodal line [C-C].

Experimental Setup and Results
The micro-channel (height of 50µm and a width of 400µm) is excited by a piezo-ceramic actuator that operates at a first mode resonant frequency of 1.88 MHz at 6.4Vpp and third mode resonant frequency of 5.59 MHz at 20Vpp, Figure 2(a). Two streams of fluids are pumped continuously into the channel while the actuator is switched between the two modes, using a relay, under a duty cycle of 50%. Figure 2(b) shows the snapshots of the beads at different time (Panels b to g). The larger 10µm sphere is identified (as shown in a circle) and traced. It can be seen that it is moved to the center node of the channel while smaller 5µm spheres remain at the lower node.
Figure 1: Particle separation using a switching ultrasound field. (a) Schematic of channel with particle trajectories of micro-spheres of different sizes (10µm and 5 µm), and the switching modes (T1=2s; T2=2s; Energy density of the first and third modes are 0.55 J/m³ and 1.2 J/m³, respectively). (b) The particles are focused within the lower critical region by controlling the relative flowrate of the two streams.

Figure 2: (a) Schematic of micro-channel and experimental setup. (b) Snapshots of the particle motion under switching acoustic field. Panel (a) shows the spheres confined within the lower region of channel before acoustic field is applied. Panels (b) to (g) show the 10µm sphere being pushed towards the center nodal line and separated from the smaller 5µm spheres.

Conclusion
A new methodology for separating two species of particles, both with positive contrast factors, is presented. This is achieved by an ultrasonic standing wave, switching between the first and third modes, that makes use of the interaction of different strengths of acoustic radiation forces (in the transverse direction) and the hydrodynamic force that pushes the particles downstream. By properly tuning the duty cycle of the switching, particles experiencing different radiation forces due to different sizes or acoustic contrast factors can be moved to different nodal lines in the channel.

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References
Elastomeric Microparticles for Acoustic-Mediated Biomolecular Separation

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Introduction
Colloids respond to an applied acoustic standing wave by transporting to specific locations along the wave (i.e., pressure node, pressure antinode). This relocation is dictated by the contrast factor which originates from differences in density and elasticity between the particle and the surrounding media. For example, particles with positive contrast (e.g., incompressible polystyrene beads, cells) in aqueous media are generally transported to acoustic pressure nodes.¹,² Here, we exploit such acoustic-mediated spatial control to develop a new bioseparation approach aimed at collecting low-quantity, targeted biological analytes from complex samples in a rapid, facile and continuous manner. This approach uses compressible particles with a negative contrast property that is opposite to commonly used microparticles (e.g., polystyrene beads and cells). In other words, negative contrast particles (NCPs) move in directions opposite from these common, incompressible particles when subjected to acoustic standing waves, thereby enabling the use of this method for bioseparation from complex samples.

Experiment
We prepared NCPs via homogenization of silicone pre-polymers in aqueous, surfactant solution using either polyvinylmethylsiloxane (PVMS) or polydimethylsiloxane (PDMS) (Fig. 1a). Because homogenization produces polydisperse particles, filtration was employed to reduce the breadth of particle size distributions (e.g., 3µm ±2µm diameters, as analyzed with microscopy). To introduce biofunctionality, NCPs were biotinylated, thereby enabling streptavidin to serve as a linker between NCPs and any biotinylated targeted analyte. We designed and fabricated microfluidic silicon devices integrated with an acoustic actuation module and a downstream separation module to enable acoustic-mediated separation and collection (Fig. 1b). The silicon devices were prepared using standard photolithography and deep reactive ion etching methods and were further fitted with a glass lid via anodic bonding to monitor separation events with microscopy. Separation experiments were monitored using a Zeiss Axio 2 microscope.

Results
Studies reveal the ability to spatially control microparticles of differing compressibility using acoustic force. Fig. 2a shows a fluorescent image of a microfluidic channel containing a random distribution of incompressible polystyrene microparticles and compressible PVMS microparticles in the absence of an acoustic field. By applying an acoustic field (2.93 MHz), separation of compressible and incompressible particles rapidly occurs (Fig 2b). Incompressible, positive contrast polystyrene particles transport to the middle of channel, corresponding to the pressure node,
whereas compressible NCPs transport to the channel periphery, corresponding to the pressure antinode.

Fig. 2. (a) Random distribution of compressible and incompressible particles within a microfluidic channel without an applied acoustic force, i.e., PZT off. The particles consist of fluorescent (yellow) polystyrene microparticles (10-14µm) and biotinylated PVMS microparticles functionalized with AlexaFluor 546 streptavidin. (b.) Separation of polystyrene and PVMS particles upon application of an acoustic field (1.98 MHz, wavelength = 2 x channel width). Fluorescent images using the red and green filters were separately obtained and superimposed for image reconstruction.

The separation of compressible and incompressible particles in Fig. 2 encouraged further investigations ultimately aimed at employing NCPs for cell separation. We hypothesized that cell-NCP complexes may transport to the pressure antinode under certain conditions. To this end, we employed polystyrene beads as cell surrogates and investigated separation characteristics. Fig 3a shows a fluorescent microscopy image of the binding between biotinylated PDMS microparticles and streptavidin polystyrene beads. These PDMS-polystyrene microparticle complexes were subsequently introduced into the acoustic-based microfluidic device. Fig. 3b shows the transport of the complexes to the pressure antinode after application of an acoustic field demonstrating the capacity for NCPs to serve in bioseparation applications.

Fig 3. (a) Fluorescent microscopy image showing binding between PDMS microparticles encapsulated with rhodamine-B fluorescent dye (red) and fluorescent (green) streptavidin polystyrene beads (6 µm diameter). Particles were incubated in a ratio of 1:10, polystyrene: PDMS for 30 minutes. (b.) The response of the PDMS-polystyrene complexes were evaluated within the acoustic-based microfluidic device without flow and with the application of 2.93MHz (wavelength = 2 x channel width).

Conclusion
We prepared biofunctional NCPs and successfully demonstrated capacity for NCPs to move rigid particles to an acoustic pressure antinode. These results encourage further pursuits aimed at using NCPs for cell separation. Advantageously, this acoustic-driven approach may enhance separation efficiency by forcing compressible and incompressible constituents in opposite directions. This rapid, continuous separation method holds potential for bio-analytical applications (e.g., rare cell and biomarker isolation, detection, analysis) and therapeutic applications (e.g., continuous bio-analyte separation from complex samples).

References
Schlieren visualization of ultrasonic standing waves in macro scale chambers

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Introduction
For the experimental characterization of optically transparent liquids and for the visualization of ultrasonic fields, the schlieren method is a proven tool². With this method spatial variations in the refractive index of the liquid are made visible. For ultrasonic particle manipulation devices particle tracking is also a very strong tool to characterize the pressure field or to visualize fluid flow. While ultimately both methods can be combined the schlieren method also offers some unique advantages. Unlike with particle tracking its timescale is not limited by drag forces and therefore is much smaller and, with the proper equipment, even allows to measure traveling waves. In addition no seeding particles are required which can influence the fluid properties such as wave damping.

Method and setup
The current setup shown in Fig. 1 a) is kept simple with a conventional (Toepler) three lens schlieren system with a first and second field lens (L1, L2) framing the object plane and a focusing lens (camera objective). The setup has been mounted vertically and the lens diameter is 50 mm. As light source a modified white LED with a 300 µm pinhole has been used. Since the devices are operated in quasi stationary mode relatively long camera exposure times can be used as compared to imaging of traveling waves. This eases the requirements for the light source and camera in terms of brightness. In the cut-off plane a knife edge filter has been used which allows to emphasize directional effects dependent on its rotational position.

Fig. 1: Schematic of vertical schlieren setup with device in the object plane and a knife stop filter a) and top view of the chamber with calculated absolute pressure field and transducer (brown) b)

The devices² investigated are made out of PMMA with a height of the fluid volume, which is the same as the assumed acoustic beam diameter L, of 3 mm and a planar top and bottom cover. They
are operated in the lower MHz frequency range with an according acoustic wavelength $\lambda$. Assuming the optical axis to be parallel to the ultrasonic wave front and an optical wavelength $\lambda_o$ of at least 400nm the Klein-Cook parameter $Q = 2\pi \lambda_o L / \lambda^2 \ll 1$ and thus Raman-Nath diffraction is expected.

With a device that uses continuous frequency sweeping, particles can be moved over larger distances using acoustic radiation forces. In macro scale chambers even a small change in frequency can change the pressure field significantly and convection as well as acoustic streaming can be of significance, thus schlieren imaging is particularly suited to investigate such a device experimentally. The device presented here is a square PMMA chamber with a side length of 20mm, excited on one boundary with a piezo electric transducer and a frequency sweep of 1.5-2.5 MHz modulated with a saw-tooth frequency of 0.05Hz.

**Results and further work**

Fig. 1 b) shows a numerical simulation in 2D of the device, where the transducer is depicted in brown and as colour graph the absolute pressure field is shown at a frequency of 1.984 MHz. The pressure field shows an inhomogeneous distribution with pressure bands along the y-axis. These bands can change their position along the x-axis significantly with a relatively small frequency change in the order of 1 kHz. These changes are often too fast and or the amplitudes and with it the primary acoustic radiation forces are too low compared to secondary forces to be studied with particle tracking. Fig. 2 a) shows a grain extract image (back minus front image plus 128 in 8-bit colour representation) of a neutral image and a snap-shot image during sweeping at 1.98 MHz obtained with the schlieren setup. The existence of such pressure bands observed in the numerical simulation can be confirmed.

Fig. 2 b) shows a grain extract image between two frames which are 50ms apart, that is a frequency difference of 2.5kHz, emphasizing effects in another time scale like caused by fluid flow. The disturbances at the lower border of the image are expected to be convection caused by the heat of the transducer close by. Some disturbances can also be caused by convection in the air, even though they should be small in a vertical setup where the airflow of rising air is parallel to the light path.

With the presented setup any kind of similar device can be investigated, e.g. devices with acoustic streaming. The quality of the imaging setup has the potential to be improved in order to record with higher sensitivity and homogeneity which can be achieved by introducing colour filters and light blocking filters of different shape or with a more sophisticated lens setup and light source.

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On the resolution and uncertainty of the Astigmatism Particle Tracking Velocimetry in acoustophoresis microchannels

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Introduction
Microfluidic chips using ultrasound standing waves for particle handling and manipulation are raising more and more interest in the Lab-on-a-chip and microfluidics community [1]. In the past few years several numerical and experimental studies have been reported with the intent to improve the understanding and the performance of future devices. In spite of the implicit three-dimensional nature of the phenomena, most experimental studies have been limited to 2D-velocimetry measurements. Consequently, detailed measurements of 3D particle trajectories and velocities in acoustophoresis devices are necessary for validation of the latest analytical and numerical predictions. The aim of this work is to provide high-resolution measurements of the 3D acoustophoretic motion of particles of different sizes, which will be achieved by using the astigmatism-particle-tracking-velocimetry method (APTV) [2].

Experiment
Experiments were carried out by an automated, temperature-controlled acoustophoresis platform with a microchip containing a straight rectangular channel of cross-section $380 \times 160 \, \mu m^2$ [3], see Fig. 1(a). Different suspensions of water and spherical polystyrene beads (with diameters of 0.5, 1 and 5 µm) were used for the experiment. For the APTV measurements, the elliptical particle images were taken using a 12-bit, 1376×1040 pixels, CCD camera (Sensicam QE, PCO GmbH). The imaging optics was formed by a M=20×, NA=0.4 microscope objective lens combined with a cylindrical lens of 150 mm focal length yielding a measurement volume of $900 \times 600 \times 120 \, \mu m^3$.

Fig. 1. (a) Schematic of the acoustophoresis microchip used for the measurement (picture from [3]). (b) Experimental arrangement for the APTV set-up. A cylindrical lens is used to obtain elliptical images of the particles. The particle image shape is directly related to the particle depth position in the channel.
To improve the quality and signal-to-noise ratio of the measurements, all beads were labeled with a fluorescent dye and epifluorescent imaging was used. A continuous diode-pumped green laser, with 2 W at 532-nm wavelength was used as illumination source. A schematic of the APTV setup is shown in Fig. 1(b). More details about the measurement method can be found in [2].

Results
The microchannel was acoustically actuated in its transverse half-wave resonance at frequency 1.94 MHz and the 3D particle trajectories were measured. For each particle size, the data were collected from a series of consecutive experiments performed at the same experimental conditions. The different behavior of particles with different size is shown in Fig. 2. Small particles (0.5 µm, Fig. 2(a)) are dominated by the viscous Stokes drag force from the induced acoustic streaming of the flow, in this case characterized by four principal macro-vortices in the vertical cross-section of the channel. On the contrary, larger particles (5 µm, Fig. 2(b)) are mainly affected by the acoustic radiation force, which concentrates the particles in the vertical center plane of the channel. The particle position was measured with an overall estimated uncertainty of less than 0.1 µm in the in-plane direction \((X-Y)\) and less than 1 µm in the depth direction \((Z)\). The instantaneous three-dimensional velocity and acceleration of the beads were computed as well from the measured path lines.

Conclusion
We used the APTV method to provide high resolution, three-dimensional measurements of beads undergoing acoustophoresis. The volumetric displacement of each bead was tracked in time, and the path lines were used to derive the instantaneous velocity and acceleration. Data were collected on polystyrene beads with different diameters (0.5, 1, and 5 µm) operating at one single resonance frequency (1.94 MHz). The high-resolution, 3D measurement by use of the APTV method opens for the development and validation of analytical and numerical predictions of acoustophoretic particle motion.

References
Spatio-Temporal Image Correlation Spectroscopy
Measurement of Microparticle Dynamics in Surface Acoustic Wave Driven Acoustic Counterflow Devices

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Introduction
In the last decade a great effort has been devoted to the development of smart integrated microfluidics pumps for portable point-of-care devices. Among them, surface acoustic waves (SAWs) nanopumps in the ultrasonic regime have recently drawn particular attention for their capability of fluid and particle manipulation [1]. It has been also demonstrated that liquid pumping into standard PDMS microchannels is enabled by SAW-driven acoustic counterflow [2].

We here study the dynamics of microparticle suspensions during SAW-driven acoustic counterflow in view of particle sorting applications. In these devices, microparticles are subjected to two major forces: the acoustic radiation force (F_{RAD}) and the Stokes drag (F_D). We characterize particle dynamics by spatio-temporal image correlation spectroscopy (STICS) and show the mean particle velocity fields. The measurements demonstrate that balance between F_{RAD} and F_D and the resulting particle dynamics are strongly dependent on the channel height.

Experiment

Fig. 1. Scheme of the microfluidic device. IDTs in front of the channel inlet are activated by an RF signal. The excited SAWs travel along the microchannel pumping the fluid from the reservoir in the direction opposite to its wavevector.

As shown in Fig. 1, SAW-driven acoustic counterflow microfluidic devices consist of two layers. The bottom layer is a LiNbO\textsubscript{3} piezoelectric substrate with two microfabricated interdigital transducers (IDTs, 96 MHz resonance frequency) for SAW excitation and detection. The top layer is a patterned PDMS film. It is composed of a 6-mm-wide square chamber and a 5-mm-long 300-$\mu$m-wide straight microchannel that extends from the centre of the chamber. A circular opening of 5-mm is punched into the chamber for fluid dispensing. Final devices are straightforwardly assembled by conformal bonding. Hybrid microchannels are finally defined by the LiNbO\textsubscript{3} bottom wall and the PDMS lateral and top walls.

30-$\mu$L particle suspension (500nm-diameter latex-based particles 2.5% in solid) is loaded in the chamber by a micropipette. We do not observe spontaneous filling of the microchannel. Pumping
IDT is then fed with 26.4 dBm continuous RF signal at 96 MHz and the suspension is actively driven into the microchannel. The filling dynamics is visualized by a transmission optical microscope and recorded at 100 fps by a CMOS-camera based acquisition system.

Results
The acquired particle dynamics is analyzed with a custom made STICS Matlab code based on [3]. Particle velocity fields for two different channel heights are shown in Fig. 2.

![Fig.2. Streamlines of the particle velocity fields during the fillings of 14-µm-high and 67-µm-high microchannels in proximity of the meniscus (a, b, d, g, i) and channel inlet (c, e, h, l). Panels a), f) illustrate the case in which the meniscus is near the channel inlet. Panels b), c), g), h) illustrate the particle velocity field when the channel is half-filled. Panels d), e), i), l) illustrate the particle velocity field when the channel is completely filled.](image)

In case of 14-µm-high channels (case h14), particles are concentrated in a periodic pattern following the meniscus profile. This accumulation pattern vanishes at 800 µm from the meniscus where particle concentration remains uniform. From this distance down to the reservoir the particles are dragged by bulk laminar flow. Vortices are present in the reservoir chamber only when the meniscus is in close proximity with the channel inlet (Fig. 2a).

Differently, in case of 67-µm-high channels (case h67) the particles follow vortex trajectories, which extend for 400 µm from the meniscus. This behaviour is found regardless the meniscus position during filling. In the proximity of LiNbO₃ floor the particles tend to accumulate in lines. As in case h14, accumulation and vortices disappear 800 µm away from the meniscus where laminar flow drives particle dynamics.

This dramatically different behaviour arises from the different balance between FRAD and FD. In case h14 FRAD overcomes FD, leading to particle concentration in the nodes of partial standing waves, as confirmed by FEM simulations. In case h67, FD overcomes FRAD. Thus, particles follow acoustic streaming vortices except than in proximity to LiNbO₃ surface, where FRAD overcomes FD owing to streaming velocity reduction. In both cases, at a distance of 800 µm from the meniscus, acoustic effects vanish and particles are subjected only to Stokes drag due to mass flow.

Conclusion
We have measured by STICS the motion of microbeads in aqueous solutions during SAW-driven acoustic counterflow in microchannels of different heights. Strong dependence of particle dynamics on the channel height is observed. We attribute this to a different balance between acoustic radiation and drag forces.

References
Separation of bacteria from red blood cells by acoustophoresis

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Introduction
Sepsis is a systematic inflammatory response to infection and the 10th most common cause of death in the US [1]. The standard method to diagnose if a case of sepsis is caused by bacteria in the bloodstream is to culture a blood sample, which may take several days. In order to shorten the time until the optimal antibiotics treatment can be started, we are working on a microfluidic system to collect and identify bacteria directly from the blood sample without culture. The first step is to separate the bacteria from the red blood cells (RBCs), which are the most numerous cells in the sample. This separation is here demonstrated using acoustophoresis in a microfluidic chip. Since the particle velocity caused by the acoustophoretic force is proportional to the square of the radius of the particle [2, 3], the larger RBCs can be separated from the smaller bacteria in the blood sample.

Methods
The experiments were performed using the chip in figure 1c. The separation channel was 27 mm long and 0.14 mm deep. The width of the channel, 0.41 mm, matches half a wavelength of the applied acoustic field at a frequency of 1.83 MHz. The channels were fabricated by anisotropic wet etching in silicon and sealed by a glass lid. Fluidic connectors and a piezoelectric actuator were then glued to the chip.

![Fig. 1. Illustration of the separation principle with the piezo actuator turned off (a) and on (b). Inlets and outlets are shown as black squares. (c) Photograph of the microfluidic chip with the white piezoelectric actuator glued beneath.](image)

The separation principle is shown in figure 1a and b. Sample is pumped into the separation channel through the first inlet from the left, at the same time as isotonic salt solution is pumped in through the second inlet, both at 200 µl/min. Without actuation, the sample is laminated along the side walls with the salt solution flowing in the center, as shown in figure 1a. When the piezo actuator is turned on, an acoustic standing wave is created across the channel with a pressure node in the center of the channel, as sketched in figure 1b. The primary acoustic force focuses the RBCs towards the center of the channel, while the smaller bacteria are less affected by the acoustic field and remain in the plasma along the channel walls. The flow is split to 1/2 in the central outlet and 1/4 in each of the...
side outlets at the end of the separation channel. The focused RBCs in the center are thereby separated from the plasma containing the bacteria.

**Results and discussion**

The chip was tested using blood from healthy volunteers, diluted to 1% in isotonic NaCl (9 mg/ml) and spiked with *Escherichia coli* DH5α. The acoustic focusing was set to the frequency with the best focusing (1.828 MHz) and the lowest driving voltage where all the RBCs seen in the microscope were focused (4.6 V_{pp}), as in figure 2.

![Fig. 2. Microscope images showing RBCs entering the chip (a), exiting the chip with the actuation off (b) and being focused with the actuation on (c). The sample is flowing from left to right and contains 10% blood for improved visibility.](image)

The output samples were analyzed by microscopy, Coulter counter, flow cytometry and PCR. 99.8% of the RBCs were removed by the separation, while 62% of the bacteria remained in the plasma, as plotted in figure 3. In addition to the RBCs, whole blood contains white blood cells (WBCs) and platelets. The WBCs were too few to be counted, but since they are similar in size or larger compared to the RBCs they are most likely focused as well. The platelets behave similar to the bacteria in the separation, which is expected since they are only slightly larger than the bacteria.

![Fig. 3. Fraction of the platelets, RBCs and *E. coli* exiting the chip that exits through each outlet, as counted in a Coulter counter. Error bars show ±1 standard deviation of three repeated measurements.](image)

**Conclusions**

The presented work demonstrates that bacteria can be efficiently separated from RBCs by acoustophoresis. This is the first step towards an acoustophoresis-based microfluidic system for fast bacteremia diagnostics.

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**References**


Synthesis of monodisperse negative acoustic contrast colloids for acoustofluidic bioseparations

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Introduction
A major bottleneck in emerging therapeutic strategies is the inability to separate rare cell types from complex mixtures. We describe a system that employs acoustofluidics to separate rare biological materials (e.g., from a standing pressure node to an antinode) by specifically binding negative acoustic contrast colloids generated by a scalable bulk synthetic approach.1

Experiment
Monodisperse silicone colloids were prepared from nucleation and growth by the hydrolysis of organosiloxane precursors bearing functional groups followed by polycondensation in a neutral or alkaline medium.2 Precursors (from Sigma-Aldrich) were combined in various molar ratios to provide precise control in colloid stability, size, compressibility, and reactivity. Colloids were then washed and characterized with DLS, optical microscopy, SEM, and zeta potential.

Results
Colloid size and compressibility were tuned by varying the ratio of di-, tri-, and tetra-functional monomers. Colloid size was tuned across two orders of magnitude (200 nm to 20 µm, Fig. 1a-e) by varying the shear rate, temperature, and catalytic reagents. Colloids were shown to exhibit positive and negative contrast when synthesized from monomers with ample and few siloxane bonds, respectively.

Fig. 1 A-C: Poly (vinyl silicone) colloids of various size. (A) Average diameter is 550 nm. (B) Average diameter is 3 µm. (C) Average diameter is 13 µm. Note: The scale bar in A is the same for A-C. Fig. 1 D-E: SEM images of poly(methyl siloxane) colloids. Colloids at a (D) 20,000x magnification and (E) 5,000x magnification.
Pink fluorescent biotin-coated polystyrene beads (from Spherotech, Inc.) were used as incompressible surrogates, representing cells (avg. dia. 5.2 µm). Negative acoustic contrast poly(vinylmethyl siloxane) colloids were synthesized vinylmethyl di-ethoxysilane (avg. dia. 13 µm). Silicone colloids were incubated in 1µM streptavidin-conjugated with Alexa Fluor® 488 (green) dye (from Invitrogen, Inc.) overnight. Fluorescent streptavidin coated poly(vinylmethyl siloxane) colloids were the positive control, Fig. 2a-b,g, pink fluorescent biotin-coated polystyrene beads were the negative control, Fig. 2c-d,h, and the two particle types bound through biotin-streptavidin binding in a 1:1 ratio were the surrogate test, Fig. 2e-f,i. These results show negative acoustic contrast colloids are capable to displace positive acoustic contrast particles from the nodes to the antinodes of an acoustic standing wave.

**Fig. 2. Acoustic separation experiment with surrogate particles.** POSITIVE CONTROL. (A) Streptavidin-conjugated Alexa Fluor® 488 incubated poly(vinylmethyl siloxane) colloids with piezoelectric transducer (PZT) power = 15V, flow = 15 µL/min. (B) PZT power = 0V, flow = 15 µL/min. NEGATIVE CONTROL. (C) Pink fluorescent biotin-coated polystyrene beads with PZT power = 15V, flow = 100 µL/min. (D) PZT power = 0V, flow = 100 µL/min. SURROGATE TEST. (E) Poly(vinylmethyl siloxane) colloids bound to polystyrene with PZT power = 10V, flow = 100 µL/min. (F) PZT power = 0V, flow = 100 µL/min. (G) Fluorescence profile along channel for positive control. (H) Fluorescence profile for negative control. (I) Fluorescence profile for surrogate test. Note: ø: PZT On •: PZT Off

**Conclusion**

We have developed a rapid approach to synthesize large quantities of monodisperse negative acoustic contrast colloids. With this system, colloids bound to rare cells have the capability to displace from a pressure node to a pressure antinode of a standing wave.

**References**

On-chip ultrasonic sample preparation

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Introduction
Sample preparation is a crucial step in many analytical and diagnostic methods. In particular, there is a growing need for fast and simple methods for isolating and up-concentrating a certain pathogen or cell from a complex sample containing a mixture of different cell types. In addition, DNA-based detection methods, such as PCR, are typically based on analysis of a lysed cell sample. Therefore, an automated, on-chip sample preparation method combining isolation/purification and up-concentration with lysis would be an attractive alternative to conventional procedures involving several steps and manual handling. A related acoustofluidic method was recently presented by Norris et al., who trapped sperm cells in a sample containing a mixture of lysed female epithelial cells and intact sperm cells [1]. However, that method was based on a separate lysis procedure off-chip prior to the on-chip cell trap.

In this paper we have combined for the first time kHz- and MHz-frequency ultrasonic actuation for on-chip size-based isolation, up-concentration and lysis of cells. As a proof-of-concept, we demonstrate MHz-frequency ultrasonic isolation and trapping of 10-µm particles from 5-µm particles, and we investigate the kHz-frequency ultrasonic lysis effect on trapped MCF-7 cells.

Experiment
Ultrasonic sized-based particle isolation and up-concentration: A modified design of the acoustofluidic chip described in Ref. 2 was fabricated and is shown in Fig. 1. A mixture of 10-µm and 5-µm particles was acoustophoretically pre-aligned in two dimensions into two nodes at combined 4.43- and 6.90-MHz actuation, followed by size-selective focusing of the larger particles into the main channel using a third transducer operating at 1.40 MHz, while guiding the smaller particles into the side channels (cf. Fig. 1). Finally, the isolated 10-µm particles were trapped and up-concentrated in an expansion chamber with a fourth transducer at 2.50-MHz actuation.

Ultrasonic cell lysis: MCF-7 cells (15-20µm) were trapped against a flow rate of 5 μm/min at 2.5 MHz and 28.5 Vpp, see Fig. 2A-B. Thereafter, a 37.5-kHz transducer was attached to the chip and operated for 3 minutes at 82 Vpp for lysing the trapped cell aggregates. In order to confirm that the cells were not lysed due to heating, we measured the temperature inside the chip, with Teflon-insulated micro thermocouple with a total tip diameter (sensor and sheath layer) of 0.41 mm (IT-21, Physitemp Instruments, USA).

Results
Separation and isolation of the beads was done as shown in Fig. 1, C, operating the transducers with the following frequency-voltage pairings: 4.43 MHz with 6 Vpp, 6.9 MHz with 10 Vpp, 2.50 MHz with 28.5 Vpp and 1.40 MHz with 10 Vpp. Finally, using confocal microscopy and the fluorescent probes calcein-AM and far-red DDAO-SE, we observed from four repetition of the experiment that
a large majority of the cells had no remaining calcein-AM in the cytoplasm after the kHz-ultrasonic treatment (see Fig. 3. A). This is one strong indication of cytolysis. The low temperature increase, shown in Fig. 3. B, indicates that acoustic cavitation is the most likely reason for the cell disruption.

Figure 1: (A) Photo of the glass-silicon-glass microchip with ultrasonic transducers. (B) Illustration of the main functions: Size-selective separation, isolation, up-concentration and lysis. (C) Microscope photos of (1) pre-alignment, (2) size-selective focusing, (3) separation/isolation, and (4) up-concentration. The sample is a mixture of green-fluorescent 10-µm particles and non-fluorescent 5-µm particles, with approx. concentration ratio 1000:1 (small particles / large particles).

Figure 2: (A) Dual-labeled (calcein-AM and far-red DDAO-SE) MCF-7 cells trapped against 5µl/min flow with a trapping efficiency of 98%. (B) Magnified image of the trapped cells with a 10X objective. (C) Confocal image of lysed cells. When the cell membrane is damaged, the green calcein-AM leaks out and only the remaining far-red DDAO is visible.

Figure 3: (A) The average number of living cells before (83%) and after (3.4%) exposure to 37.5-kHz ultrasound at 82 Vpp, average and standard deviation from 4 experiments. (B) The graph shows the increase in temperature during three minutes of ultrasonic exposure at 37.5 kHz and 82 Vpp.

Conclusion
Our measurements demonstrates that an acoustofluidic chip operated with five different ultrasonic transducers (between 37.5 kHz and 6.90 MHz) can be used for on-chip sample preparation, by performing in a sequence the following cell manipulation functions: Pre-alignment, size-selective separation, isolation, up-concentration and lysis.

References
Controlling non-inertial cavitation microstreaming for application in biomedical research

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Non-inertial cavitation microstreaming is a steady fluid flow generated by time averaged Reynolds stresses resulting from viscous damping in the boundary layers of oscillating gas bubbles [1]. In this paper, progress is reported towards the development of a device in which controllable and repeatable microstreaming flows can be used to mechanically stress biological cells. Mechanical stress has been reported to temporarily porate cell membranes, which is useful for targeted drug delivery [2]. Moreover, mechanical stress may lead to the expression of specific genes in cells that are exposed to fluid flows in-vivo (e.g. endothelial cells exposed to blood flow [3]). The majority of literature reports on the use of lethal inertial cavitation to stress cells however, the flow velocity is seldom measured [4]. Within the research presented here, much gentler stable cavitation microstreaming is utilised, the velocity of which is characterized using particle image velocimetry (µPIV).

Figure 1: (a) The microfluidic device in which experiments are carried out. (b) A vector field of microstreaming around an Expancel microbubble. The top left vector is a reference vector equal to 50 μm s⁻¹.

Microstreaming was generated inside a purpose built microfluidics device (figure 1.a) by an acoustic standing wave. A single planar pressure anti-node was generated inside a 200 μm tall fluid channel within the device. The wave was setup using a PZT piezoelectric transducer with an area of approximately 1 cm², driven at a frequency of 555 kHz and with a range of acoustic pressure amplitudes which were modelled to be of the order of 100’s of kPa. Commercial gas filled, hollow microspheres (Expancel®) were incorporated into PDMS, forming the lower surface of the fluid channel. H9c2 cardiomyocytes were cultured on to the opposite face (the acoustic reflector) which was then clamped into place with magnets just prior to beginning of each experiment. A µPIV system was built for capturing image pairs and used to obtain flow velocity fields (figure 1.b). For the purpose of µPIV measurements, the flow was seeded with 1μm fluorescent beads.
Experiments carried out inside the microfluidic device have demonstrated that toroidal fluid vortices are generated around Expancel microbubbles and that they are highly stable even at velocities on the scale of millimetres per second. The relationship between the driving voltage and the microstreaming velocity can be described by simple polynomial functions. Microstreaming has been found to be highly dependent upon the resonance of the microbubbles and also on the spatial position of the microbubbles within the acoustic chamber. The latter being a direct consequence of inhomogeneity in the pressure field.

The effect of microstreaming on the viability of adhered H9c2 cardiomyocytes was measured in order to assess the suitability of stable microstreaming flows as a method for mechanically stressing cells. The rational for this is that if flow velocity can be controlled then the extent of cell damage, or indeed the preservation of cell viability can also be controlled and would thus be useful in a variety of biomedical applications. Cell viability was measured by incubating cells with an intracellular fluorescent probe, CellTracker™ Orange (Invitrogen), prior to ultrasound exposure and then trypan blue staining following ultrasound exposure.

Microstreaming in the specifically designed device was found to be repeatable in the vicinity of each microbubble however there were large variations in velocity between microbubbles. The fixing of microbubbles in a thin layer of PDMS has proven to be a suitable method for increasing controllability and repeatability of the microstreaming. By culturing cells onto the acoustic reflector and subsequently sealing them in the device, it has been possible to demonstrate that the shear stress generated was of a useful magnitude for application to biomedical research.

References
Acoustic radiation torque for rotation of non-spherical particles

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Introduction
Ultrasonic manipulation is commonly used to move and position spherical particles. Additionally, the contactless rotation of particles is possible with ultrasonic standing waves. The acoustic radiation torque and the viscous torque can both be used to spin a particle. The viscous torque is generated by a near boundary streaming due to two orthogonal phase shifted standing waves. This torque can be used for the rotation of spherical objects. Non-spherical objects can be rotated by the acoustic radiation torque generated by a standing pressure wave. There exists already quite a number of publications and applications for other contactless particle manipulation techniques like dielectrophoresis, magnetic or optical manipulation. This is one of the motivations to explore the acoustic radiation torque on non-spherical particles in ultrasonic manipulation devices. The rotation of particles can be useful in lab-on-a-chip systems or for micro robotic ultrasonically assisted assembly processes.

Acoustic radiation torque
The acoustic radiation torque acts on non-spherical particles like a fiber exposed to an ultrasonic standing wave. Objects smaller than a quarter wavelength will be aligned at the pressure nodes [1]. Therefore a finite element simulation was used to predict the torque on a non-spherical particle. Beside the torque, the equilibrium position of the object could be estimated. The equilibrium position and torque are depending on the size and material properties of the non-spherical object.

For the development of rotation techniques the Gor’kov potential was used to predict the equilibrium position of a short fiber. The finite element simulation was used to verify this approach. The model consists of a 3D acoustic domain (\(\lambda/2\times\lambda/2\times\lambda/4\)) where a solid fiber is placed inside. Fig.1a shows the absolute pressure distribution for a two dimensional excitation in x- and y-

![Fig.1](image)

**Fig.1** a) Absolute pressure distribution of the acoustic domain surrounding the fiber (depicted in white). b) Acoustic radiation torque \(T_z\) acting on the fiber plotted over the angular position \(\alpha\) of the fiber (frequency 1 MHz, fiber length 200 \(\mu\)m), computed from the first order pressure distribution by means of a perturbation approach.
direction and the position of the fiber. The resulting time averaged radiation torque $T_z$ (plotted in Fig.1b) is depending on the angular position $\alpha$ of the fiber and is computed from the first order pressure distribution by means of a perturbation approach. The torque was derived for a glass fiber (length 200 $\mu$m, diameter 15 $\mu$m) suspended in water with an excitation frequency of 1 MHz and a maximal pressure amplitude of $1.6 \times 10^5$ Pa. The equilibrium position of a fiber for the shown pressure distribution in Fig.1a is at an angular position of 45° and 135° where the torque equals zero, whereas the 45° position should be unstable.

Methods for rotation

Different methods have been developed which can be used for the rotation of non-spherical objects. A simple method is the alternating generation of standing waves with difference in the propagation direction. This can be realized with a hexagonal shaped cavity [2]. Here, we will focus on the possibilities of the superposition of orthogonal standing waves. A varying pressure field with change of orientation of the corresponding potential force field can be generated with the amplitude modulation of two superimposed orthogonal standing waves. The rotational velocity and the direction of rotation is determined by the frequency of the amplitude change. Every angular position of an object can be obtained by a certain set of amplitudes. Also the phase modulation of superimposed orthogonal standing modes can lead to a rotation. Therefore the excitation or geometry of the device have to lead to an asymmetry and to a slight resonance frequency difference between the two orthogonal standing modes.

Experiments

The experimental verification of the above described methods have been performed with the device depicted in Fig. 2. A 3×3 mm$^2$ fluidic chamber has been etched into silicon and covered with glass. The excitation is done by a 4×4 mm$^2$ piezoelectric transducer fixed on the silicon plate directly underneath the fluidic chamber.

Fig. 2 a) Back side of the micro-device showing the piezoelectric transducer with the defined strip electrodes. b) Micro-device with the fluidic chamber and the cone shaped inlet channels. c) View of the fluidic chamber with a glass fiber (length 200 $\mu$m) suspended in water. d) Rotation of a glass fiber with phase modulation at a frequency of 1158 kHz. The 6 different fiber positions have been extracted from video frames and are equally spaced by 0.3s.

An experiment is shown in Fig 2.c, where a glass fiber (length 200 $\mu$m, diameter 15 $\mu$m) suspended in water can be seen. The fiber starts to rotate like shown in Fig. 2d by applying the phase modulation method. Therefore one of the strip electrodes of the piezoelectric transducer was set to a frequency of 1158 kHz and another strip electrode was excited with a slight frequency difference of about 0.35 Hz. The slight frequency difference will lead to a phase modulation. The direction of rotation can be controlled by choosing the second frequency slightly above or below 1158 kHz. The frequency difference is determining the rotational velocity.

Acoustophoresis of full and hollow particles in two-dimensional resonance modes

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Introduction

Acoustophoresis is developing from one-dimensional standing waves towards manipulation in higher dimensions. For example, acoustic particle traps and biological particle assays require two-dimensional acoustic mode shapes. So far, most two-dimensional approaches cover the ultrasonic micromanipulation of full particles or cells in a superposition of one-dimensional standing waves \cite{1,2,3}. In this work, we extend these approaches by experimental and analytical studies of the behaviour of hollow particles in superposed as well as intrinsically two-dimensional resonance modes.

Methods

For experimental evaluations, a 1.2 mm square chamber was etched 200 \textmu m deep inside a silicon microdevice as shown in Fig. 1. Driven by a piezoelectric transducer with patterned electrodes, the behaviour of suspended particles in the fields of the fluidic chamber was microscopically observed for various excitation modes.

Results

As illustrated in Fig. 2, particles in a square fluidic chamber could be aligned in a single centred clump. For full particles, an approach with frequency switching was shown in Fig. 2a, whereas for hollow particles in 2b, a different two-dimensional mode shape was required. The acoustic radiation forces in this two-dimensional resonance mode were found to be highly dependent on particle characteristics.
On the analytical side, for hollow particles the effect of the inner particle radius on their Gor’kov potential [4] in Fig. 3a was analysed. The weighting of pressure and velocity field influences on the particles was found to be depending on the compressibility and density of hollow particles. We have plotted the acoustic radiation force on hollow particles as shown in Fig. 3b with a match between analytical and numerical calculations.

**Fig. 2.** Trapping of particles (~17 µm) in the center of the 1.2x1.2mm fluidic chamber. (a) For full solid particles, approaches with frequency switching and modulation were successful, 636/682 kHz. (b) Hollow particles were attracted to the pressure- as well as velocity nodes in the chamber, which lead the particles to the center for this two-dimensional resonance mode at 870 kHz.

**Fig. 3.** (a) The Gor’kov potential field $U(x,y)$ for hollow particles in a two-dimensional resonance mode. The acoustic radiation forces $F$ are predicted to point to the center for particles with lower average density than the surrounding fluid. This behaviour was observed in the corresponding experiment of Fig. 2b. (b) The normalized acoustic radiation force is plotted over the ratio of inner and outer radius of a hollow particle. As the matching analytical and numerical calculations reveal, not only the magnitude, but also the sign of the force is dependent on the inner particle radius.

**Conclusion**

Novel strategies for the two-dimensional particle alignment in square chambers were described with relevance for applications as particle traps and assays. The special acoustic properties of hollow particles showed to be beneficial for a specific resonance mode shape, whose influence on particles is dependent on their acoustic characteristics. This finding is also of interest in the converse argument for particle characterization.

**References**


Introduction
The aim of the project is to isolate stem cells from peripheral blood samples using acoustophoresis. The stem cells are harvested from mobilized donors or patients using a clinical apheresis instrument, which produces an apheresis product containing white blood cells, stem cells and platelets. The acoustophysical parameters of stem cells are too close to the white blood cells to enable separation, so in order to extract the stem cell fraction affinity beads (4.5µm Dynabeads) are envisioned to be used. The bead combined with the targeted cell results in a larger compound, with significantly different acoustophoretic mobility, and hence can be sorted acoustophoretically. However, as the number of stem cells present in the apheresis product is quite low, ~1%, and the antibodies are quite expensive, an experimental model using CD4+ T helper cells was used in the initial development of the method. The number of CD4 cells is much higher, about 15-20% of the total mononuclear cell count, which facilitates the experimental conditions in the development phase.

Experiment
Magnetic beads that targeted T helper cells (CD4+) were added to the apheresis sample and the beads were bound to the targeted cells in an incubation step. The magnetic beads were used to be able to compare the acoustic separation method with the standard magnetic bead based separation technique (MACS) for stem cell separation. An acoustophoretic chip with 2D pre-focusing abilities was used for the separation of the bead tagged cells, depicted in figure 1. The pre-focusing of the sample in two dimensions enabled all cells to have the same starting positions in the parabolic flow profile when entering the main separation region. This allows an optimal tuning of the acoustic force for separation as the retention times for all cells in the channel are the same. After the pre-focusing step, the cells enter the separation channel laminated against the channel walls and are then exposed to a 1D acoustic standing wave which will move the bead bound cells into a new cell free buffer and will consequently exit through the center outlet. The wash buffer is comprised of pure Ficoll solution, a medium much denser that the PBS based sample solution, and enhances the separation of the beads from the unwanted white cell population.
Figure 1. Sample suspension enters from the left and passes through the pre-focusing region which moves all cells and beads into two pressure nodes. As the sample progresses into the separation channel, the denser magnetic beads are transferred into the new Ficoll buffer present into the center of the channel. The mononuclear cell population is not as affected and exit through the side outlets.

**Results**

Results show that acoustic sorting purities of $90 \pm 7\%$ (n=10) were achieved, compared to $94 \pm 5\%$ (n=10) purity obtained with control magnetic bead sorting (Figure 2). The slight decrease in performance of the acoustic system is probably due to that the amount of beads bound to the targeted cells are not homogeneous, and the magnetic system is less sensitive to the bead bounding number as the magnetic force is much higher as it relies on magnetic properties rather than size. The average viability of acoustophoresis sorted cells ($94 \pm 5\%$, 7AAD staining) was also comparable to the magnetic sorted cells ($97 \pm 2\%$, 7AAD staining).

Figure 2. The performance of the acoustic separation compared to the magnetic separation. As can be seen in the graph, the magnetic performance is slightly higher than the acoustic. This is probably due to the magnetic separation being less sensitive to the number of beads bound to each target cell.

**Conclusion**

The separation of CD4+ cells (T helper cells) with affinity beads using acoustophoresis is possible and the change to a stem cell targeted affinity bead (CD34) should be feasible.
Acoustic Focusing and Separation of Nanospheres using Microfluidics

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Introduction
Separation and concentration of nano-sized particles are important for many biological analyses. For example, separation of nano-sized particles conjugated with an affinity molecule of interest will be useful in diagnostics and potentially separations. It further offers opportunities to develop novel microfluidic chip based bio-analytical tools for clinical and non-clinical applications. Concentration of stationary nano-particles has been demonstrated [1]. Manipulation of flowing particles in nanometer size range via ultrasonic standing waves has been assumed to be difficult due to the weak force experienced by such small particles as acoustic theory predicts focusing power is proportional to size of the particle [2]. While there are many examples of successful movement of µm diameter particles using ultrasonic standing waves, this work explores the lower size limits of concentration of flowing nanoparticles via ultrasonic standing waves and presents the acoustic focusing particles as small as 200 nm in microfluidic channels at varying flow rates.

Experiment
Microfluidic flow channels used in this work are etched in silicon substrate using standard photolithography and deep reactive ion etching techniques. The microfluidic channel consists 5 cm long simple straight channel and it is trifurcated into 1 cm long channels at the end (Figure 1). The etched channel is enclosed by anodic bonding a Pyrex™ glass slide on the top of the channel. Samples are introduced to the channel via silicone tubing inlet attached to a drilled hole on the glass slide at non-split end. Three-silicone tubes are attached as outlets at the trifurcated end. Resonant standing waves are generated via a single lead zirconate titanate (PZT) transducer attached to the bottom of the device. Microfluidic channels are designed to create a single focused stream at frequencies of either at 2.91 MHz or 4.48 MHz. Acoustic focusing of polystyrene and silica nanospheres with varying sizes and surface chemistries are evaluated against different flow rate and acoustic force conditions. For nanosphere separation applications, nanospheres are focused into the middle of the channel and samples from three outlet arms are collected as three separate fractions and measured for the particle concentration in each fraction.

Fig. 1. Top (left) and side (right) views of the microfluidic acoustic device used in the work. Here a 2.91 MHz PZT with the dimensions of 30 mm x 5 mm x 1 mm is attached to the bottom of the assembled device to create a focused stream of nanoparticles.
Results
In our initial studies, we have been able to focus particles in the range of 200 nm - 500 nm. Theory on acoustic focusing suggests increasing particle density will improve particle focusing. We observed this while focusing silica and polystyrene nanospheres at similar experimental conditions, where we observed better focusing of silica nanospheres than that of polystyrene nanospheres at low applied voltage and higher flow rates. We analyze the extent of focusing via fluorescence images collected via epi-fluorescence microscopy (Figure 2, left) and their line scanning (Figure 2, right). We will further present analysis of separation efficiency via fluorescence spectrophotometer. We will also present our findings with regards to differing chemical treatments on polymeric nanospheres that affect acoustic focusing of the particles.

![Fluorescence images and line scanning data](image)

**Fig. 2.** Epi-fluorescence images of focused 500 nm silica nanospheres flowing at a flow rate of 140 µL/min (Left, top) and 10 µL/min (Left, bottom). Line scanning data of focused and non-focused streams of silica nanospheres flowing at varying flow rates (Right).

Conclusion
In this work, we have shown that it is possible to focus particles as small as 200 nm in diameter. We are able to focus nanospheres at flow rates up to 140 µL/min. We anticipate that nanosphere focusing can be enhanced by material composition, flow channel improvements, as well as improved energy coupling into our current device. We will present estimates of the smallest effective particle sizes that can be manipulated using routine ultrasonic standing wave devices. We will also present general conclusions on how chemical treatments might be used to provide improved manipulation of biological and polymeric nanoparticles.

References

Numerical modelling of thermo-viscous effects on acoustic radiation forces on an elastic particle

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Radiation forces are non-zero, time averaged forces acting on a particle in an acoustic field. If second-order terms are retained when the pressure is integrated over a control surface of the sphere and time-averaged, then the result is the acoustic radiation force (first-order terms average over time to zero). This force can be used to trap, separate, filter, or mix particles in a host fluid [1]. A detailed calculation of the acoustic force on a rigid sphere in a plane standing or progressive wave field in an ideal inviscid fluid was first presented by King [2]. Since then significant work has been published on estimating acoustic radiation forces including compressibility [3], non-spherical shapes [4, 5], elasticity [6] and viscosity [7] but the scattering functions required to derive analytical solutions effectively limit them to simple geometries and sizes. Numerical formulations have allowed arbitrary geometries to be considered, but these have typically been limited in their representation of the particle or field characteristics [8, 9], or have been very computationally demanding [10], and have generally approximated to non-viscous conditions to simplify the model [11]. This approximation is reliable for particles of dimension larger than the thickness of the acoustic boundary layer [7]. Recently, an analytical formulation was presented using viscosity and estimating the force in a far field condition in the long wavelength limit [12].

In small devices particles are often close to the wall and it is important to study the effect on a particle near the wall. Recently work was reported for predicting forces near a wall using an analytical and numerical method [13] but this is limited to a cylindrical particle and an inviscid fluid. We developed here a numerical formulation including thermo-viscous effects and study the interaction between an elastic particle and the reflecting wall in a standing wave field.

We have extended our previous FEA perturbation model [11] to include the thermal and viscous properties of the host fluid (Figure 1(a)). We have implemented a similar approach to Setnes and Bruus [12] in our model and considered a “viscous region” that is five times the depth of the acoustic boundary layer and treated the remaining field as inviscid. The estimated radiation force has been compared in a far-field condition with [12] where the approximation for the 2nd order radiation stress tensor [13] is valid due to the absence of viscosity. We have verified the limitation of inviscid solutions and confirm the effect to be only significant for small particles. Further, a modified approach provides the flexibility to predict the radiation force in the near field, on the particle boundary. Consequently, the model is used to analyse the effect of proximity to a wall. As shown in figure 1(b) the particle is placed close to the pressure maximum near the wall and remaining boundaries use non-reflecting boundary conditions. The results are compared with Wang and Dual’s results [14].
for inviscid conditions near a wall. The results suggest that the radiation force is stronger near a flat wall in the viscous model than in the inviscid model and forces are also more sensitive to the shape of the particle in the viscous case.

Figure 1: (a) 2D Axi-symmetric model showing the velocity distribution near the non-slip boundary wall (b) 2D Axi-symmetric model particle close to pressure antinode near the wall

Particle and bacteria aggregate structures in acoustic levitation

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The purpose of this study is to demonstrate the possibility of manipulating particles and bacteria in order to generate structured aggregates. By using particles of different acoustic impedances it’s possible to generate an axial segregation, leading to a multilayered structure i.e., multilevitating structure. This multilevitation effect can be the first step of a separation process when the system is coupled to transversal flow. Those manipulations could be achieved by carefully modifying the resonance frequency and wave amplitude. In this study, we shall present the manipulation of particles of different acoustic properties i.e. latex particles and porous silica particles both of 10 µm in a 250µm thickness resonator.

Fig 1. a) Aggregate mixture of latex and silica beads of 10 µm in acoustic levitation at the same plane. b) Latex particles remain at the levitation plane, while silica particles aggregate levitate separately at a certain distance in the depth. Multilevitation is obtained. 10X magnification.

We can manipulate and generate structures with bacteria (Escherichia coli) in acoustic levitation. Under the forces generated by standing waves, we can obtain thin layers or bacteria aggregates according to their intrinsic mobility and concentration. Competition between hydrodynamic, acoustic and biological interactions may generate solid, liquid and gas-like structures. Mixture with particles allows visualizing such effects.
Increasing throughput in microchannel acoustophoresis – A design study

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Introduction
Acoustophoresis is emerging as an alternative technique for processing biological samples in medicine\(^1\), food\(^2\) and environmental applications. In order to be useful in this context, the throughput must be sufficiently high. A clinically relevant sample size of 10 mL must be processed within a relevant time, typically less than an hour, indicating flow rates of ~150 µL/min or higher. Sample dilution (typically 10-20 times) before processing is many times needed, which puts further requirements on high throughput and push flow rate requirements up to several mL/min. Samples can be processed faster, with higher throughput, by increasing the acoustic primary radiation force by applying more voltage to the piezo actuator. However, by increasing the voltage the temperature of the piezo element rises and hence the acoustophoresis chip will pose a limit in the ability to process biological samples that are often sensitive to heat. Optionally, the sample throughput can be increased, without increasing the primary radiation force, by extending the retention time of the sample in the acoustic pressure field through increasing the length of the acoustophoresis channel. This abstract presents a study on two different designs of extended microchannels and considerations to be made for each design. A primary requirement is that the channel design is chip area conservative and can be operated at \(\geq 1\)mL/min.

Experiment
Two different designs of ~30 cm long microchannels, with one inlet and one trifurcation outlet, were fabricated in glass (Fig. 1). The designs were made to be surface conservative, \(i.e.\) maximising the channel length in a given surface area. This reduces the relative amount of bulk material per channel length and we believe thereby that we can minimise the relative energy loss. The microchannels were 150 µm deep and 375 µm wide. Divided by 300 µm thick walls they covered ~2 cm\(^2\) excluding the inlet and outlet regions. 8 µm diameter polystyrene beads were used to characterize the chips.

Results
The longer channels allow for acoustic focusing at higher flow rates. Figure 2 shows 8 µm beads focusing in the meander channel. As they flow through the channel the beads get more and more focused, as we move to the right in the picture. However, when introducing a longer or curvilinear channel geometry together with the given channel dimensions and a higher flow rate, effects of the inertial forces and Dean flow will be prominent. In both designs these effects can in themselves focus beads. In the meander they have a positive effect on partially focused or unfocused beads. However, when combined with fully acoustically focused beads these will be partially defocused after...
each curvature and is thus dependent on retention time, i.e. channel length, to refocus before the next curvature or the outlet. This problem is not found in the spiral channel as the channel only changes turn direction once, when passing through the spiral centre. The inertial forces together with the Dean flow will in the spiral channel shift the acoustically focused beads from the channel centre towards the spiral centre (Fig. 3). In both channel designs the inertia also causes the focused bead band to split into two 2D focused bands situated over each other (Fig. 4). Flow rates above 1 mL/min with well focused particles were observed for both channel designs. More detailed separation data and flow rate dependency will also be presented.

Conclusions
When designing a longer high-throughput acoustophoresis microchannel a surface conservative chip will have lower energy losses. In this abstract a meander and a spiral acoustophoresis channel design have been studied. In both channel designs the effects of inertial forces and Dean flow can be seen. This causes e.g. a partial defocusing of the acoustically focused band in the curvature of the meander channel and the focused band to shift its position towards the centre in the spiral design. Both designs are promising in terms of high-throughput displaying well focused bead streams using 8 µm beads at flow rates ≥ 1 mL/min; although the spiral design is currently giving the most promising results.

References
Acoustic Actuated Fluorescence Activated Cell Sorting

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Background
Fluorescence activated cell sorters (FACS) are great workhorses in laboratories today. Detection and sorting of fluorescently labeled cells is performed at rates of 100,000 cells/s. While excelling at sorting speed, most FACS instruments offer few possibilities of adding additional features. A µ-chip based FACS solution has been suggested to address this limitation [1]. The lab-on-a-chip (LOC) approach enables handling of small volumes with high yield. Up- or downstream functionality such as cell incubation, parallel processing, PCR or other assays can also be integrated. In addition to these LOC benefits, the acoustic approach offer online analysis and versatility in terms of sample handling and buffer conditions.

Methods
Acoustophoresis is a contact free method for manipulating cells and particles in microchannels [2]. By actuating a microfluidic channel with ultrasound at its resonance frequency, a standing wave is formed. A suspended particle in an acoustic standing wave is affected by an acoustic radiation force, which over time will move the particle to either a pressure node or to an anti node depending on the acoustic properties of the particle [3].

Based on the acoustophoresis principle, we have developed an acoustic actuated FACS (AFACS) that has the potential to work in the kHz regime. The principle of the AFACS is illustrated in Figure 1.

All particles are first two-dimensionally aligned by the ultrasonic radiation force under continuous flow in the microchannel. This “prefocusing step” improves the optical detection, accuracy and throughput. Upon detection of fluorescent particles, the sorting zone of the chip is actuated with 2MHz ultrasonic bursts, deflecting particles enough to allow capture of these in the target outlet. The throughput and sorting purity of the system is limited by how fast a particle can be deflected from the pre-aligned position to a position where it will exit through the target outlet. The microchip is fabricated in Si [100] using anisotropic wet etching and
anodic bonding to a borosilicate glass lid. The Piezoelectric transducers are glued to the back of the chip.

**Results**
Fluorescent and non-fluorescent 10µm polystyrene microparticles were sorted in 9 experiments, where throughput and the ultrasonic actuation time were varied. At least 10,000 particles were sorted per experiment. Samples were analyzed using a Bürker chamber before and after sorting. The experimental results are presented in figure 2.

![Figure 2](image_url)

**Figure 2.** Experimental result from 9 experiments where the ultrasonic actuation time and throughput has been varied.

**References**
An analytical calculation for the acoustic time-averaged phenomena around an infinite cylinder in a viscous fluid

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Introduction
It is well known that the time-averaged phenomena, including the acoustic radiation force and acoustic streaming, arise around a particle exposed in a sound wave. These interesting phenomena derive from the nonlinear convectional effects of the medium containing sound waves and are distinct very much from the periodic ones in the linear acoustics. The time-averaged acoustic effects have many engineering applications [1] such as ultrasonic particle manipulations, acoustic levitation and mixing and so on. In order to improve the efficiency and the accuracy of those acoustic devices, it is necessary to formulate these time-averaged phenomena in detail for further physical understandings. Many pioneers, e.g. King [2], Gor’kov [3], Riley [4], Doinikov [5], Wang [6] and Bruus [7], have addressed this topic under specific conditions. Most of the former investigations were focused on axisymmetric acoustic forces acting on a sphere or a cylinder in an inviscid (King and Gor’kov) or a incompressible (Riley) and a compressible viscous (Doinikov, Wang and Bruus) fluid. In this paper, an analytic general formulation for the time-averaged acoustic phenomena for an infinite cylinder is established based on the perturbation method under the conditions of small incident amplitude. The solution is more general and accurate than Wang [6]. Moreover, it can also be extended to the three-dimensional situation. The formulation presented here provides more insightful physical understanding to the time-averaged acoustic force and may become a theoretical basis for numerical modeling to compute the acoustic force in practical cases such as irregular particle shapes and complex device boundaries.

Theory
The first- and second- order dimensionless governing equations for a viscous flow motion without heat conduction are

\[ M^2 \frac{\partial \rho}{\partial t} + \nabla \cdot \mathbf{v}_1 = 0 \]

\[ \frac{\partial \mathbf{v}_1}{\partial t} = -\nabla p_1 + \frac{1}{M^2} \left( \nabla^2 \mathbf{v}_1 + \frac{1}{3} \nabla (\nabla \cdot \mathbf{v}_1) \right) \]

with the linear equation of state \( p = \rho \), where the dimensionless parameter \( M^2 = \frac{\omega^2 a^2}{c_s^2} \), indicating the compressibility and \( M^2 = \frac{\omega a^2}{\nu} \) denoting the viscous effects.

Linear field
The first-order are equivalent to the following equations by introducing \( \mathbf{v}_1 = -\nabla \varphi + \nabla \times \psi \mathbf{e}_z \)

\[ (\nabla^2 + k_s^2)\varphi = 0 \quad \text{and} \quad (\nabla^2 + k_s^2)\psi = 0 \]

with the boundary condition \( \varphi = \frac{\mathbf{u}_i}{m_j} \int S \cdot \mathbf{dS} \), where \( \alpha_i \) is the first-order stress and \( m_j \) is the particle mass. The solutions for Eq. (2) are

\[ \varphi = \sum C_n \left[ I_n(kr) + \alpha_n H_n^{(1)}(kr) \right] e^{i\omega t} \quad \text{and} \quad \psi = \sum C_n \beta_n H_n^{(1)}(kr) e^{i\omega t} \]

where \( C_n \) are the expanding coefficients of the incident wave and \( \alpha_n \) and \( \beta_n \) are the scattering coefficients determined by the boundary conditions.
Time-averaged field

By introducing $\mathbf{v}_2 = \nabla \times \Psi e_z$ and $M^2 \mathbf{F}_g = \nabla \times \mathbf{Q} e_z$, we have

$$\nabla^2 \Psi = -Q \quad \text{and} \quad \rho_2 = -\frac{1}{M^2} q$$

(4)

with the boundary condition of the inner flow as $\Psi = \Psi' = 0$ at $r = 1$ and $\Psi' = \text{constant}$ when $r \to \infty$. By assuming

$$\Psi = \sum_n \Psi_n(r) e^{i\omega t}, \quad q = \sum_n q_n(r) e^{i\omega t} \quad \text{and} \quad Q = \sum_n Q_n(r) e^{i\omega t}$$

(5)

One can obtain the solution of Eq. (4), for example $n = 1$ as

$$\Psi_1 = -\frac{r^4}{16} \left( \int x^2 [i\ln b_1(x) - b_3(x)] dx + C_{1,1} \right) + \frac{r^3}{4} \int (\ln x b_1(x) + (1 + \ln x) b_3(x)) dx + C_{1,3} + \frac{1}{2} C_{2,2}$$

(6)

where the parameter $b_{1,n}$ and $b_{2,n}$ are defined by $r M^2 F_g = \sum (e_n b_{1,n} + e_n b_{2,n}) e^{i\omega t}$. The coefficients are determined by the boundary conditions as

$$C_{1,1} = \int x^2 [i\ln b_1(x) - b_3(x)] dx, \quad C_{2,2} = \int (\ln x b_1(x) + (1 + \ln x) b_3(x)) dx, \quad C_{5,3} = -\frac{1}{2} C_{1,1} \quad \text{and} \quad C_{6,1} = -2C_{2,2} - C_{1,1}.$$  

The other solutions for $n < -1, n = -1, n = 0$ and $n > 1$ can be obtained in the same manner.

Acoustic streaming and force

The solution such as Eq. (6) presents the acoustic streaming velocity in the boundary layer. The velocity at the top of the boundary layer ($r \to \infty$) can drive a streaming out of the boundary layer, which can be solved easily. The time-averaged forces acting on a rigid cylinder per length in $x$- and $y$- directions are then calculated by

$$F_x = \pi \text{Re}[\sigma_{2n+1} + \sigma_{2n+1} + i\sigma_{2n-1} - i\sigma_{2n+1}] \quad \text{and} \quad F_y = \pi \text{Re}[i\sigma_{2n-1} + i\sigma_{2n+1} + \sigma_{2n+1} + \sigma_{2n-1}]$$

(7)

where $\sigma_{2n+1} = \sigma_{2n-1} - \sigma_{2n-1}$ and $\sigma_{2n+1} = \sigma_{2n-1} - \sigma_{2n+1}$. The parameters $\sigma_{2n+1}$ and $\sigma_{2n-1}$ are determined by the second-order velocity achieved in the last subsection and $\sigma_{2n+1}$ and $\sigma_{2n+1}$ are determined by the time-averaged first-order momentum flux with $\langle \mathbf{v}_1 \times \mathbf{v}_1 \rangle_{\omega} = \sum \sigma_{2n+1} e^{i\omega t}$ and $\langle \mathbf{v}_1 \times \mathbf{v}_1 \rangle_{\omega} = \sum \sigma_{2n+1} e^{i\omega t}$.

Example

A rigid cylinder of radius $5 \times 10^{-5}$ m is fixed in the host medium with the density $1000$ kg/m$^3$ and the speed of sound $1400$ m/s. The incident planar traveling wave is of density amplitude $0.1$ kg/m$^3$ and a frequency of $2.8$ MHz. The acoustic radiation force is given as $8.787 \times 10^{-4}$ N/m in an ideal fluid by using the theory in Ref. [8]. The total time-averaged force predicted by the current theory including viscosity is $8.879 \times 10^{-4}$ N/m which is close to the results in Ref. [6].

References


Optimization of acoustic perturbation in microfluidic device for biological and biomedical applications

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Introduction

On-chip acousto-microfluidic systems are flexible and accessible components being explored for a variety of applications including flow cytometry, bioseparation, cellular trapping and colloidal manipulation. In the design of the most efficient and low cost devices for specific applications, we still need to address several fundamental issues, e.g., dimensionality and stability, energy dissipation, and sensitivity to the resonant condition. We hereby present our modelling and experimental work, where new hypotheses of the asymmetry of the perturbation, energy dissipation and stability are posed and tested. The linear wave equation and acoustic radiation force are solved in the 2-dimensional limit, and verified via confocal microscopy.

Theory and Experiment

Figure 1 shows the fabricated device and provides a schematic illustration of the cross section with four periodically oscillatory liquid-solid interfaces. These perturbations at the interfaces are necessary to induce pressure wave propagation inside the channel in 3-dimensions. Since the viscous force overrides the acoustic radiation force in flow direction, we will confine our modelling work to 2-dimensions. At the lowest resonant mode for a channel with a square cross section, i.e., $W = H = \lambda/2$, the pressure is given by Eq. 1.

$$p_x(x, t) = \left[ (Z_L U_R e^{i\varphi_R} - Z_R U_L) \cos(kt) + i\rho_0 C_0 (U_L - U_R e^{i\varphi_R}) \sin(kt) \right] e^{i\omega t} \quad \text{Eq. 1a}$$

$$p_y(y, t) = \left[ (Z_R V_T - Z_T V_B e^{i(\varphi_B - \varphi_T)}) \cos(kt) - i\rho_0 C_0 (V_T - V_B e^{i(\varphi_B - \varphi_T)}) \sin(kt) \right] e^{i(\omega t + \varphi_T)} \quad \text{Eq. 1b}$$

where $k$, $Z$, $\varphi$, $U$ and $V$ represent the wave number, boundary impedance, phase shift of these perturbations, velocity amplitude in $x$-direction and in $y$-direction, respectively; $\rho_0$ and $C_0$ represent the density and sound propagation speed of water. Our model suggests that in phase perturbations result in the efficient formation of high amplitude standing waves with mid-channel pressure nodes in the lowest resonant mode.

In experiments, we have tested two typical acoustic driving strategies, where a piezoelectric transducer (PZT) is aligned and bonded either underneath the channel or to an etched-through trench aside from the channel. Confocal microscopy revealed the 1D and 2D focusing of colloids under the two different driving strategies, and also compared the efficiency for each strategy. Suspensions of Nile red labeled polystyrene beads (10 μm, Spherotech, Inc.) were imaged under a Zeiss LSM 780 upright confocal microscope with a $z$-resolution of 4 μm / frame.
Introduction

Optimization of acoustic perturbation in microfluidic devices for biological and biomedical applications still need to address several fundamental issues, e.g., how to control the acoustic radiation force in flow direction, we will confine our modelling work to the lowest resonant mode.

Results

As shown in Fig. 2a, stability is determined by the pressure in both the transverse (x) and vertical (y) directions. Fig. 2c shows that the particles are simultaneously focused in x and y directions when the channel PZT is activated. Fig. 2d suggests that, since the air-pocket is apart from the channel, the focusing in the y-direction is poor. The quality of the focusing depends on both of resonant condition and residence time of particles (e.g., cells) through the channel.

Conclusion

In phase perturbation induces acoustic standing waves with high efficiency. Furthermore, a single PZT can induce acoustic waves in multiple dimensions which may have advantages in certain applications. For a particular application, the driving strategy and design of the microfluidic channel should be deliberately selected. For example, for bioseparations of particles to different streamlines (i.e., values of x), the recommended design is $W = \frac{3}{4} \lambda_x$ and $H = \left( \frac{5}{2} + \frac{1}{4} \right) \lambda_x$. For flow cytometry, on the other hand, a square cross section is appropriate where the particles are pushed into a tight space towards the center from all directions.

References

Influence of the substrate of multilayer systems on the acoustic field establishment inside their channels of treatment

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Introduction
The properties and nature of all the walls in resonating microchambers and cavities of multilayer resonators play an important role in the establishment of the standing waves within them. The spatial distribution of the acoustic pressure generated in 2D standing waves is strongly influenced not only by the boundary conditions defined by the walls between which the wave is established but also by the other walls, as found in this work. In particular, this influence has been numerical and experimentally analyzed in polymeric 3D resonators, whose substrate and lid play a key role in the transmission of the acoustic wave toward the cavity of treatment, behaving as a 3D vibrating structure or sometimes as a surface waves generator depending on the relationship between the materials of both, the substrate and the top containing the channel.

Experiment
Experiments were carried out using 3D multilayer resonating chips, whose scheme is presented in Figure 1.

Fig. 1. Scheme of the whole device that includes the PZ ceramic attached to the lateral edge of a polymeric chip including the channel of treatment and another parallel channel closed with air

In some of them the PMMA substrate was replaced by pyrex to analyze the particle behavior inside the channel of treatment. Aqueous suspensions with polystyrene 20μm-sized spheres were subjected to the acoustic field at different resonant frequencies close to 1MHz.

Results
The particles showed different behaviors in both chips, collecting at different locations at each of these frequencies, as shown in the filmed images of Figure 2, obtained at f=1020kHz.

The use of pyrex replacing the PMMA substrate make inhibites the majority of 3D vibrations produced within it, with the consequent influence on the wave transmitted toward the cavity of work, acting as a surface wave generator.
A numerical modelling has been performed using Comsol Multiphysics to analyse the structural vibrations in different polymeric chip designs as well as the acoustic field distribution introduced inside the channel of treatment. The simulation includes the whole piezoelectric element attached to the polymeric chip containing the channel of treatment with the samples. A module of piezoelectricity has been included to combine piezoelectric elements and purely mechanic elements defining the piezoelectric Material Model for the piezoelectric ceramics and Linear Elastic Model for the rest of materials. The study includes an acoustic-structural mechanical coupling at the interface between the piezoelectric actuator and the polymeric chip, as well as at the solid-liquid interfaces in the channel.

At a frequency $f=1020$kHz the study predicts a 3D structural vibrations shown in Figures 3 and 4 for PMMA and pyrex substrates respectively, with the pressure distribution inside the channel of both chips given by Figures 3.b and 4.b.

**Conclusion**

Numerical and experiments confirm the influence of the substrate material as a 3D vibrational structure or generator of surface acoustic waves depending on its stiffness, which is relevant for any optimization of chips with microchannels for particle/cell manipulation.
Dexterous manipulation of microparticles using circular ultrasonic arrays

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The ability to trap and manipulate objects on the micro-metre scale has attracted interest in the biosciences and for micro-fabrication. While the use of optical scattering to apply forces, with optical tweezers, is well established, analogous systems using ultrasound are in their relative infancy. Previously the authors have used two matched opposing pairs of transducers to produce a movable grid of traps in water. The current work extends this approach to allow individual traps to be created and manipulated independently using a circular ultrasonic array.

For small dense particles in water the effect of a standing acoustic field is to force the particles away from antinodes in the pressure field and towards nodes. This can be exploited by generating fields with nodes surrounded by regions of higher pressure amplitude to form traps. A device capable of producing, and updating in real time, arbitrary fields in a fluid filled chamber will allow dexterous manipulation of multiple traps. Arrays allow the generation of arbitrary fields over a region determined by the pitch of the elements (aliasing degrades control outside this region). Using a circular array, forming the periphery of the fluid chamber, results in a region of control whose radius is proportional to the number of elements. The presence of resonances in the device chamber complicates the control problem, and this is addressed using analytical and finite element models.

A prototype device has been fabricated by backing and dicing a piezoceramic ring into 16 elements. The device is operated at 2.4 MHz (the thickness mode of the elements) to maximise pressure amplitudes. The trapping and manipulation of an agglomerate of 10-micrometer-diameter polystyrene particles was demonstrated with this device.
Separation of particles in a multi-wavelength macro-channel using an ultrasonic acoustic standing wave

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Introduction

In this work, separation of 37 μm size soda lime glass sphere particles from water in a flow channel 25mm wide using an acoustic standing wave is reported. Ultrasonic acoustic standing wave separation technology has been widely used in different micro-scale applications in the areas of biotechnology and drug delivery [1], [2]. There are few works performed to utilize ultrasonic acoustic pressure at the macro-scale where single particle theory is challenged [3]. In this paper, the applicability of a water-matched piezo transducer to a 25mm × 25mm cross section channel is investigated and the transverse velocity of particles shifting to pressure nodes is measured and compared to single particle theory.

Experiment

A schematic and digital image of the experiment are shown in Figure 1. The setup is based on a flow channel with a test section comparable to a previous static setup reported in [4]. The experiment is carried out in a flowing channel connected between two tanks containing a mixture of degassed distilled water and 37 μm soda-lime glass spheres with the specific gravity of 2.5 gr/cm³. The volume concentration of particles is calculated to be ~0.1 % by image processing.

Figure 1: (a) The schematic of the flow channel. The water-matched piezo transducer (diameter $D_t = 30mm$) is driven by a continuous sinusoidal wave ($V_{pp} = 22volts$). The illuminator system including a 532 nm laser and a two mirror scanning laser sheet maker is illuminating the particles through the glass slide which is also acting as an acoustic reflector. (b) The photograph of the experimental flow channel with the three main components of the setup. A 500 kHz resonance frequency, water-matched high-power piezo transducer forms one side of the test section of the flow channel and is excited with a continuous sine wave produced by a function generator. The other side of the test section formed by a glass slide acts as the acoustic wave reflector. Images of particles in a thin light sheet from a 532 nm laser are captured using a high resolution camera and long working-distance microscope. The images are processed using a hybrid particle image velocimetry (PIV) (DaVis 8, LaVision GmbH) technique to determine particle velocities.
Results
Figure 2 shows the results of separation of particles and PIV analysis of particle motion due to the primary acoustic force. The PIV analysis is performed by optimizing processing parameters and results have been collected over five tests, each one containing 300 pairs of double frame images. In Figure 2(a), a typical particle field image is shown while the fluid is flowing top-to-bottom. At the top of the image, dispersed particles are observed. Particles are agglomerated in the middle of the field-of-view where the acoustic wave is applied. Horizontal velocities averaged over different height locations are shown in Figure 2(b) and compared with theoretical values using the pressure magnitude of the wave as a fitting parameter. As it is shown, there is a good agreement in the pattern of the simulated scaled horizontal velocity and the experimental values.

Conclusions
In this paper, motion of particles under the influence of an acoustic standing pressure wave is investigated using PIV. Accounting for the viscous drag force using the Stokes drag equation and the primary acoustic force, particle motion is simulated and compared with the experimental values. The results show that the model is capable of predicting the pattern of the motion of the particle in a single acoustic wavelength.

References
Cell viability measurements during ultrasound-related stimuli may provide insight into mechanisms involved in the phenomena of contrast agent-free sonoporation

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Introduction

Sonoporation, the use of ultrasound (US) to create transient pores in biological cell membranes, is traditionally undertaken in the presence of contrast-agent microbubbles (CA). Although CAs drastically enhance the formation of membrane pores [1], they are also known to compromise cell viability [2] due to their associated oscillations and cavitational activities. Moreover, CAs add complexity to the elucidation of the mechanisms involved in membrane poration since it is difficult to discriminate CA-induced bioeffects from those of US alone. In our previous study [3] we demonstrated CA-free sonoporation of a cardiac myoblast cell line whilst maintaining high cell viability, utilising ultrasonic standing wave (USW) fields within microchannels. Here we expose cells to “gentle-to-aggressive” US-related physical stimuli in order to identify boundaries between conditions that significantly reduce cell viability and conditions that create only transient stress. This may facilitate understanding of the mechanisms governing transient membrane stress associated with sonoporation.

Experimental

The sonoporation system comprised of a squared cross-section, borosilicate glass capillary (length: 50 mm, internal width: 300 µm and wall thickness: 150 µm), acoustically coupled to a piezoelectric transducer and mounted on a glass platform (Fig. 1). An USW was generated within the device and a resonant frequency sweep (2.13–2.40 MHz) established. H9c2 cardiac myoblasts (2×10⁶ cells/mL) were suspended in DMEM culture medium and infused into the device using a syringe pump (continuous flow, Qin = 2.59 mL/h), exposing cells to US for a nominal 5 sec.

Fig. 1. Schematic of CA-free sonoporation microdevice, consisting of a glass capillary coupled to a PZT transducer, mounted on a glass platform. L: transducer length; IW: capillary internal width; t: capillary wall thickness.

The effects of the following physical parameters on cell viability were investigated: (i) US amplitude with varying peak-to-peak voltage in the range of 0-29 Vpp, at 0.05 sec fixed sweep interval. Flow visualization experiments using fluorescent tracers and numerical simulations were employed to characterise the acoustic environment and the onset of streaming at varying Vpp. (ii) Temperature (T) variation due to heat generated by the PZT. Thermal effects were evaluated by immersing the device into a thermostatic bath in the absence of US. Fluid temperatures corresponding to PZT surface temperatures at different Vpp were assessed. Computational fluid dynamics (CFD) simulations were performed to predict and quantify fluid temperature fields within the capillary. (iii) Frequency sweep duration varying in the range of 0.02-0.35 sec at fixed Vpp.
Results

Fig. 2 demonstrates that cell viability was not compromised by US exposure up to ~15 V<sub>pp</sub> at a sweep interval of 0.05 sec. A ~17% reduction in cell viability was measured at 21 V<sub>pp</sub>, corresponding to the onset of Rayleigh-like streaming (observed with fluorescent tracers), while a ~60% reduction was measured at 29 V<sub>pp</sub>, corresponding to an observed onset of “chaotic” streaming. Fig. 3a shows cell viability in the presence and absence (control) of US. During US the PZT temperature was noted to increase from ambient (23°C) to 56°C, at 29 V<sub>pp</sub>. However, CFD simulations (Fig. 3b-c) revealed that the temperature of the liquid medium within the capillary increased from ambient to a maximum of 34°C. To validate this, control experiments at 56°C revealed that cell viability was maintained at 79.8±8.9%, while under US exposure viability reduced to 35.7±5.5%.

![Fig. 2. Effect of peak-to-peak voltage (V<sub>pp</sub>) on H9c2 cell viability (%) at fixed sweep interval of 0.05 sec.](image)

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![Fig. 2. Effect of peak-to-peak voltage (V<sub>pp</sub>) on H9c2 cell viability (%) at fixed sweep interval of 0.05 sec.](image)

**Fig. 3.** (a) Effect of temperature on cell viability (T=56°C), either in the presence (US) or in the absence (NO US) of ultrasound. (b) Temperature profile along the capillary centreline, measured computationally. (c) Fluid temperature contours measured computationally (black line: capillary centreline). (d) Effect of sweep interval on cell viability, at a fixed V<sub>pp</sub>=21.

![Fig. 3d reveals that changing the sweep interval (T<sub>s</sub>) resulted in detectable variations of cell viability. At a fixed V<sub>pp</sub>=21 and T<sub>s</sub>≥0.12sec high cell viability was maintained and no significant variation in cell viability was observed by increasing the sweep interval. However, at T<sub>s</sub><0.12sec cell viability decreased with reducing sweep interval, with significant reduction in cell viability (~20%) detected at T<sub>s</sub>≤0.05sec.](image)

**Conclusion**

The effect of individual US-related physical parameters (US amplitude, fluid temperature and sweep interval) on H9c2 cell viability was assessed within a microfluidic channel. High cell viability was maintained at amplitudes where streaming was not evident. However, when streaming velocities increased, cell viability significantly reduced. In addition, cell viability was minimally affected by PZT-generated heat. Longer duration frequency sweeps were identified to have little or no effect on cell viability, whereas short sweeps resulted in reduced cell viability. Our experimental findings have allowed discrimination between conditions that significantly reduced cell viability and conditions that created only transient stress, thus potentially facilitating our understanding of the mechanisms governing CA-free sonoporation.

**References**

Particle collection at air-liquid interfaces

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Introduction
Particle and cell clustering in distinct patterns is demonstrated on the free surface of microfluidic volumes. Employing ultrasonic actuation, submersed microparticles are forced to two principal positions: nodal lines of a standing wave within the liquid bulk, and distinct locations on the air–liquid interface (free surface). The fundamental mechanisms behind such patterns have been unravelled, and it is shown that the contribution of fluid particle velocity variations on the free surface results in patterned particle clustering. In addition, by varying the size and density of the microparticles (3.5 – 31 µm polystyrene and 1 – 5 µm silica), acoustic streaming has been found to increase the tendency for a smaller particles to cluster at the air–liquid interface. This selectivity is exploited for the isolation of multiple microparticle and cell types on the free surface from their nodally-aligned counterparts.

There are two notable works on free surface clustering using vibration, in both cases the effect is caused by low frequency capillary waves (<100Hz). Falkovich et al. demonstrated the collection of hydrophobic and hydrophilic floating particles at the velocity anti nodes and nodes, respectively, of a low frequency standing capillary wave on a free liquid surface [1]. In addition, surface acoustic waves have been used to excite both ultrasonic waves and, through complex subharmonic generation, low frequency resonances. This results in the formation of particle island assemblies, located on the free surface of a droplet [2]. In the ultrasonic regime, efforts to date have focussed on particle manipulation within the liquid bulk. However, the ability to cluster on the free surface offers numerous benefits to many applications, including on-chip detection and self-assembly procedures, and could significantly improve on-chip detection (such as via Raman spectroscopy [3]) as the presence of other constituents and the fluid suspension would not impede imaging.

Experiment
The device used for experimentation consisted of a 0.5 mm thick piezoelectric element (Pz26, Ferroperm, Denmark), 0.5 mm thick silicon wafer, and fluid chamber (0.5 by 3 by 4 mm). Upon injection of a 6 µL particle/cell suspension the device was actuated using an AC signal supplied by a signal generator (Stanford Research Systems DS 345, USA) and a power amplifier (Amplifier Research 25A250A, USA). Particle behaviour was viewed and captured using an upright microscope (Olympus BX51, Australia) with additional illumination (Edmund Optics MI-150, USA) coupled to a video camera (Hitachi HV-D30, USA).

Results
Upon actuation of the microfluidic device, such that a standing pressure field was setup within the open chamber, two distinct particle responses were observed, as can be seen in Fig 1 (a). The first is the commonly encountered particle alignment at pressure nodes, which has been well chartered within closed microfluidic chambers. At a frequency of 1.53 MHz, five nodal lines were established across the shortest chamber dimension (equispaced at 0.8 mm). The second observed response comprises the clustering of particles in distinct patterns at the air–liquid interface. The four clusters form in the internode spacing, with a small amount of clustering at the walls of the device. Notably, both particle types (polystyrene and silica), regardless of their surface characteristics, were found to cluster at the same locations on the free surface. This is in direct contrast to the low frequency capillary wave effect in which, hydrophobic and hydrophobic particles would be driven to different locations within such a standing capillary wave. To determine if the location of the clustering (within the intermodal spacing on the free surface) is a general finding, a 2.1 µL droplet was actuated at frequencies causing complex nodal patterns (f = 1.296 and 1.490 MHz). The results show that the intermodal spacing location is preserved, Fig 1 (b). Finally, Fig 1 (c) shows the potential to
selectively capture particles on the free surface, with 6 and 31 μm particle types being isolated from one another.

The ability to collect particles at the free surface using ultrasonic actuation can be seen from examination of the acoustic radiation force field potential (Fig 2 (a)) which results when a zero pressure boundary condition is imposed on the upper fluid surface (and excitation is selected at a resonant frequency). It can be seen that collection is predicted at the nodes on the lower surface of the fluid. It can also be seen that at the air-liquid interface force potential minima occur at locations above the pressure maximum. Hence, there are clusters at the upper surface in between the collection on the lower surface. The ability to separate between particle sizes is due to the acoustic streaming, which tends to pull the particles out of the nodal alignment, as depicted in Fig 2 (b). This occurs preferentially for smaller particles as the downward acting acoustic radiation force, which must be overcome, scales with radius cubed, whilst the streaming induced drag forces scales with radius.

Conclusions
Ultrasonic pressure fields established in open fluid volumes are capable of collecting particles on the air-liquid interface, as well as the pressure nodes within the fluid. Due to the additional effect of streaming this occurs in a manner such that particle selectivity can occur. Furthermore, applications could be found in on-chip detection, and self-assembly, exploiting the fact that submerged particles can be driven to the interface for monolayer assembly as defined locations.

References
An Interdigitated Microelectrode Array for Capture and Lysing Cell as a Tool for Downstream Analysis of Acoustofluidic Cell Separation

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Introduction

In the past five years, acoustofluidic technologies offer rapid and label-free particle or cell separations with high resolution and have been attracting considerable interests [1, 2]. To analyze the separated cells, additional functions should be integrated to the acoustofluidic platforms. In this paper, we present an interdigitated microelectrode array for capture and lysis of target cells to analyze a gene expression level with RT-PCR. The intracellular mRNAs of target cells are obtained by lysing cells with electroporation (EP), where micro-sized electrodes allow high electric field strength with low electric potential. Efficiency of EP is successfully increased by capturing target cells to the edges of electrodes with DEP (Dielectrophoresis), which allows us to apply high electric field strength on the cell membranes. RT-PCR was successfully demonstrated with the lysate obtained on the present microelectrode array. Our microelectrode array will be integrated with acoustofluidic cell separation platform as its downstream analytical component.

Experiment

Figure 1 shows the fabricated interdigitated microelectrode array for capturing cells by DEP and lysing cells by EP and its experimental setup used in this study. Interdigitated microelectrodes made of ITO (Indium Tin Oxide) with 6-µm gap are patterned on a glass substrate (Fig. 1a) and a PDMS chip with 2-mm hole as reservoir is bound onto the substrate as shown in Fig. 1b. As a demonstration, capture and lysis of U937 cells (Human leukemic monocyte lymphoma cell line) stained with Calcein-AM (Wako Pure Chemical Industries Ltd.) were conducted on the microelectrode array. The cells were dispersed in a DEP buffer (10 mm HEPES, 0.1 mm CaCl₂, 59 mm D-glucose and 236 mm sucrose; pH 7.35; 21.4 mS m⁻¹) for positive DEP. To capture the cells, 3 Vp-p sinusoidal electric potential at 1 MHz was applied to the electrodes. To lyse the cells, a series of electric pulses of 100-µs long, 300 V high were applied for around 10 times.

Fig. 1. (a) Microphotograph of the interdigitated electrode array with the PDMS chip. The scale bar is 500 µm. (b) Photograph of the experimental setup.
After the lysis, RT-PCR was conducted using a commercial kit (Takara One step SYBR PrimeScript PLUS RT-PCR Kit, Takara Bio Inc., Japan). As a target of RT-PCR analysis, an 80bp sequence on CD45 (leukocyte common antigen) gene was selected. To avoid RNA and DNA contamination, an oxygen plasma treatment (50 sccm, 20 Pa, 75 W, 5 sec) against the substrate was performed by using a reactive ion etching machine before the cell capture. The temperature conditions for the RT-PCR for CD45 gene were set as follows; reverse transcript: 45°C (5 min), and for the PCR: 95°C (5 s), 58°C (30 s) and 72°C (30 s) for denaturation, annealing and extension, respectively. The PCR is implemented with 40 cycles and the inactivation of reverse transcriptase was conducted by the incubation at 95°C for 10 s before starting PCR process. The RT-PCR products were separated on a 3% agarose gel.

**Results**

Figure 2 shows the fluorescence images of capture and lysis of the U937 cells on the fabricated interdigitated microelectrode array with the PDMS chip. As shown in Fig. 2a, the cells were successfully captured at the edges of the microelectrodes by DEP force. Captured cells were lysed by EP. The cell lysis was verified by monitoring the release of Calcein-AM inside the captured cells caused by the disruption of cell membranes (Fig. 2b). Since the cells were captured to the edges of electrodes with DEP, we can apply high electric field strength to the captured cells and efficiently disrupt the cell membranes. The cell lysate of 1 µl (roughly containing $6.0 \times 10^2$ cells) in the reservoir on the chip was collected using a pipette and the following RT-PCR process was conducted using a thermal cycler. The resultant image of gel electrophoresis is shown in Fig. 2c. It indicates that the size of the RT-PCR products (80 bp) from the cell lysate obtained by EP (lane 3) is the same as the products from the cell lysate obtained by a conventional cell lysis buffer as positive control (lane 2). We are presently investigating about the smear of DNA shown in lane 5.

**Fig. 2.** Cell capture by DEP force and cell lysis by EP. (a) Cells were captured at the edges of microelectrodes by DEP force. (b) Cell lysis by electroporation. The scale bars are 500 µm. (c) Result of gel electrophoresis of RT-PCR products. (1) 25-bp DNA ruler, (2) RT-PCR products from the cell lysate obtained by a conventional cell lysis buffer as positive control, (3) RT-PCR products from the cell lysate obtained by EP, (4) Negative control for (2), (5) Negative control for (3).

**Conclusion**

In this paper, we report an interdigitated microelectrode array for cell capture and lysis for RT-PCR analysis. We believed that this microelectrode array has potential to be used as a tool for downstream analysis of acoustofluidic cell separation. As a next step, we will integrate a microchamber array [3] on to the electrode array to compartmentalize captured individual cells and perform single-cell RT-PCR analysis.

**References**


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