Imaging the position-dependent 3D force on microbeads subjected to acoustic radiation forces and streaming
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Imaging the 3D force distribution on micro beads subjected to acoustic radiation forces and streaming†

Andreas Lamprecht,*a Stefan Lakaemper,a Thierry Baasch,a Iwan A.T. Schaap,b and Jurg Duala

Acoustic particle manipulation in microfluidic channels is becoming a powerful tool in microfluidics to control micrometer sized objects in medical, chemical and biological applications. By creating a standing acoustic wave in the channel, the resulting pressure field can be employed to trap or sort particles. To design efficient and reproducible devices, it is important to characterize the pressure field throughout the volume of the microfluidic device. Here, we used an optically trapped particle as probe to measure the forces in all three dimensions. Via automated scanning routines, spatial variations within the volume of the channel were imaged. We found that multiple fabricated devices showed consistent pressure fields. Forces in the direction of the standing wave resulted in a periodic energy landscape with an effective stiffness for acoustically trapped $2.06 \, \mu m$ beads of 2.6 nN/a. Surprisingly, forces perpendicular to the direction of the standing wave reached values of up to 20% of the main-axis-values. To separate the direct acoustic force from secondary effects, we performed experiments with different bead sizes, which attributed some of the perpendicular forces to acoustic streaming. This method to image acoustically generated forces in 3D can be used to either minimize perpendicular forces or to employ them for specific applications in novel acoustofluidic designs.

1 Introduction

Contactless manipulation of immersed objects by sound or light provides a powerful addition to the toolbox of microfluidic engineering. It offers active means to bias processes that are otherwise purely driven by fluid dynamics. Here, the use of ultrasonic standing waves (USW) has received considerable attention, as it allows the manipulation of small spherical objects (like functionalized micro-particles), and extends to differently-shaped objects, like rods and shells. Relevant for biomedical and diagnostic applications are the manipulation of droplets, hydrogel particles serving as pico-liter containers, and of living organisms, ranging from bacteria and individual cells to whole organisms.

The basic physical principles governing acoustic manipulation are well understood under ideal conditions and a series of publications of the acoustofluidic community summarizes the current knowledge. Briefly, by choosing the right frequency an acoustic standing wave can be created between the walls of a microchannel generating one or more pressure nodes in the channel. Due to acoustic radiation forces, particles with an appropriate acoustic contrast will move to these nodes that act as a potential well. If the acoustic pressure field $p$ and velocity field $u$ of the standing wave field is known, the interacting force on a particle was derived by Gor’kov for a compressible particle in a non-viscous fluid.

In practice, several aspects complicate the design of acoustic flow cells. i) Acoustic streaming is a continuous flow of liquid within the acoustofluidic device which results from the absorption of acoustic energy primarily at the viscous boundary layer $\delta$ at the solid-liquid interfaces. In a flow cell that accommodates multiple pressure nodes, such as ours, the acoustic streaming is predicted to form multiple vortices (Fig1a). For spherical particles the drag force that is exerted by the streaming liquid scales linearly with $r$ (Stokes’ law). This different scaling behavior as compared to the acoustic radiation force makes smaller particles more sensitive to streaming than larger ones, whose response will be dominated by the radiation forces.

In contrast to streaming, the radiation force results from the direct acoustic field and thus, linearly increases with the size of the particle. Additional forces that have been observed are the acoustic pressure force and the acoustic streaming force acting in the direction of the wave. These forces are a result of the acoustic field and thus do not depend on the particle size. They result in a drag force that is exerted by the pressure gradient and the velocity field. These forces can be expressed as $f = \frac{1}{2} \rho V^2 c^2 k r^2$, where $\rho$ is the density of the fluid, $V$ is the velocity of the particle, $c$ is the speed of sound in the fluid, $k$ is the wave vector and $r$ is the radius of the particle.

In this study, we performed experiments with different bead sizes, which attributed some of the perpendicular forces to acoustic streaming. This method to image acoustically generated forces in 3D can be used to either minimize perpendicular forces or to employ them for specific applications in novel acoustofluidic designs.

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to the direction of the intended standing wave. iii) Particles will be subjected to additional forces due to scattering of the acoustic waves by other particles. These scattered waves from other particles and backscattering from the walls of the flow cell will lead to additional acoustic radiation forces that can occur in all three dimensions.

For the directed development of applications in small-scale acoustic microfluidic devices, a better understanding of the aforementioned aspects is important. Although numerical simulations have been able to capture many features of acoustic manipulation\(^7,8\), a full agreement with measurements is often still looming. Experimental characterization of pressure-distributions remains essential to identify contributions from acoustic radiation, streaming, and scattering effects in newly designed acoustic devices. Because the forces that act on the particles will not exclusively be oriented along the acoustic waves but have perpendicular components as well, it is important to include all force-vector components in the measurements (i.e. \(F_{xyz}\)), to gain a complete representation of force variations throughout the channel volume.

Over the last few years, a couple of experimental studies have sought to determine acoustic forces within acoustofluidic channels. Barnkorb et al.\(^14\) systematically inferred the time-averaged acoustic forces on moving particles in 2D from microparticle image-velocimetry (PIV) and derived pressure amplitudes by feeding the data into existing models. This work was extended by 3D microscopic observations of moving small beads in acoustically formed streaming patterns inside an acoustofluidic channel\(^13\). Thalhammer et al.\(^15\) used an optical trap to displace a particle within an acoustic standing wave field, away from its equilibrium position, and obtained the 1D force from the particle-motion back to equilibrium by PIV\(^14\). Bassindale et al.\(^16\) used multiple holo-graphically generated optical traps and video-based particle tracking at 150fps to compare the forces in 2D on particles at defined positions within an acoustic standing wave\(^15\). In a recent publication, Oever et al.\(^17\) presented an elegant probe-free method for determining the acoustic pressure distribution using a stroboscopic, tomography-like method\(^17\). This method nicely visualized the 2D \(xy\) pressure distribution averaged in \(z\)-direction within acoustic chambers for particle free systems. In a proof-of-principle study\(^18\), we recently employed the spring-like properties of a single-beam optical trap and video-based particle tracking to measure time-averaged acoustic forces in 2D at multiple positions along the standing wave. The acoustic pressure amplitudes derived from radiation forces were independent of the size of the probing particle and were consistent with values from numerical simulations.

While all of the aforementioned achievements have made significant contributions to a better understanding of the acoustic pressures and resulting forces, a method that measures the acoustic force components in all three directions throughout the volume of the flow cell has been lacking. Here, we present an integrated experimental approach to directly measure \(F_{xyz}\) at any position in the acoustic flow cell. Our approach is based on a single beam optical trap to hold a micrometer-sized bead that is used as probe to detect gradients in pressure. The resulting force will push the bead out of its equilibrium position in the trap. The force vector is obtained by measuring this displacement and multiplication with the spring constants of the optical trap. Detection of the displacement in all three dimensions is done by two decoupled quadrant photodiodes, one optimized for \(xy\)-detection and one for \(z\)-detection\(^19\). Automatic routines were used to control the motorized microscope stage in \(xy\) and a piezo \(z\)-scanner to map out the volume of the flow cell in 3D while automatic calibrations and measurements were performed at each position. The use of different sized particles allows to separate the measured total force \(F_{xyz}\) into its acoustic radiation force components and its Stokes drag components due to acoustic streaming.

## 2 Materials and methods

### 2.1 Optical trapping setup

The optical tweezers are based on the apparatus described in detail elsewhere\(^20\). The output of a 285mW, 785nm near infrared fiber coupled laser diode (FPL785S, Thorlabs, USA) is collimated by a lens system and coupled into a standard microscope chassis (Nikon NI-U, Tokyo, Japan), where the optical trapping potential is formed by a high numerical aperture (NA) water immersion microscope objective (CFI Plan Apo IR SR 60XWI 1.27NA, Nikon, Japan). Immersol W (Zeiss, Germany) is used as an immersion medium between the lens and the investigated acoustofluidic flow cell to avoid evaporation during the measurements. Downstream of the trapped particle, the laser light is collimated by an air condenser (Nikon, NA 0.9) and split into two separate beams by a non-polarizing 50:50 beam splitter (CCM1, Thorlabs, USA), as shown in detail in Fig. 1b. At each outlet of the beam splitter, a quadrant photo diode (QPD)(Thorlabs, PDQ80A, US) is mounted at a plane that was the optical conjugate of the back focal plane of the microscope objective. The optics were designed such that one QPD was optimized for detecting the \(z\)-displacement by restricting the NA of the light path\(^21\). The second QPD was optimized to detect the \(xy\)-displacement by using an unrestricted NA. The four analog signals of the QPDs are first converted to positional signals by the built-in analog electronics before low-pass anti-aliasing filtering at 15kHz. The data was digitized by a data acquisition board (NI USB-6356, National Instruments, Austin, TX, US) and the recorded signals were processed by custom-written LabVIEW and MatLab routines for calibration and force measurements in 3D. Calibration of the position (\(\mathbf{P}\)) and force sensitivity (\(\mathbf{F}\)) was performed using a conventional passive calibration method\(^21,22\). Briefly, a 10 second signal of the recorded Brownian particle motion is transformed into a power spectrum and the calibration constants were obtained via the equipartition theorem\(^22\). Calibrations were performed for the \(x\)-, \(y\)- and \(z\)-directions. The values for the optical trap stiffness \(\kappa\) in \(x\)- and \(y\)-direction had comparable values, while the stiffness \(\kappa\) in \(z\)-direction was about an order of magnitude weaker. Such an axi-symmetric trapping potential is also expected from the elongated shape of the diffraction limited focal spot in the \(z\)-direction. Typical optical trapping stiffness (80 mW laser power) for a 2.06\(\mu\)m silica particle was 1.6\(\times\)10\(^{-6}\)\(\frac{N}{m}\) in the \(xy\)-plane and 0.2\(\times\)10\(^{-6}\)\(\frac{N}{m}\) in the \(z\)-direction. The larger 4.39\(\mu\)m silica particles had a typical optical trapping stiffness (180 mW laser power) of 3.3\(\times\)10\(^{-6}\)\(\frac{N}{m}\) in the \(xy\)-plane and
0.3 \cdot 10^{-6} \frac{N}{m^2} \text{ in the } z\text{-direction. The laser power was chosen to achieve a trap stiffness that was high enough for the particle to remain in the linear optical regime under the highest acoustic force that appeared in our experiments. Positioning of the optical trap in the } xy \text{ direction within the acoustic flow cell was performed by a closed-loop motorized microscope stage with a step size of 10 nm (SCAN, Marzhauser, Wetzlar, Germany). Positioning in the } z\text{-direction was performed by a closed-loop piezo stage with a resolution of 0.75 nm (PI, P-725.2CD, Karlsruhe, Germany). The force repeatability of our measurement setup was better than } \pm 11\% \text{. This value was obtained by repeating the same measurement 51 times at a fixed position and same frequency sweep (3-4 MHz) for a total of 240 minutes. This value includes positional drift and eventual variations in temperature. }

2.2 Ultrasonic device

The acoustofluidic devices (Fig. 1c) were fabricated in a cleanroom-facility to assure well defined geometries and sharp channel walls, and are a further development of the device described by Lakaemper et al.\textsuperscript{18}. In brief: a glass-silicon-glass device was processed by GESIM (Grosserkmannsdorf, Germany). A 1 mm thick 4in glass wafer forms the bottom layer of the device and a 120 \mu m mono crystalline silicon wafer was combined with the bottom glass by anodic bonding. The thickness of the silicon layer defined the channel height. The channel was created by etching through the entire silicon layer in a photolithography process step. Two different devices with respective channel widths of 2 and 4 mm were created. A 250 \mu m glass wafer was bonded on the silicon layer and forms the top layer of the channel. The glue-free design helped to obtain an acoustic quality factor \( Q = \frac{f}{\Delta f} \) of 375 at \( f = 3819 \text{kHz} \) (resonance), due to the low dissipation losses at the bonded glass-silicon-glass interfaces. To increase the range of the optical trap all the way to the bottom of the channel, the thickness of the cover glass was reduced to 140 \mu m by an additional HF etching step. The transparent channel design ensured the possibility to use the QPD position detection in the experiments. The layered device was cut to 76x22 mm with the fluid channel in its center. The resulting channel dimensions (w x h x l) were 2x0.12x76 mm and 4x0.12x76 mm. The piezoelectric transducer (Ferroperm, Pz26, l x w x h = 8x2x0.5 mm, Kvästra, Denmark) was glued on the thin top glass layer by conductive glue (EPOXY Technology, H20E, Billerica, MA, US). The orientation of the transducer was parallel to the channel wall at the center of the channel length. The distance from the center of the piezoelectric element to the middle of the channel width was 9 mm, which was necessary to avoid collision with the microscope objective. The transducer had its first thickness eigenmode at 4.00 MHz. The sinusoidal drive signal was generated by a function generator (Tektronix, AFG 3033B, Beaverton, OR, USA) and a power amplifier (MKS Instrumente Deutschland GmbH, ENI 2100 RF, Berhausen, Germany) set at 10 V_{pp}.

2.3 Experimental procedure

To achieve reproducible and accurate results the measurement steps were largely automated using LabVIEW (National Instruments, Austin, Texas, US) routines. First, the device was filled with pre-filtered water (< 0.25 \mu m) and monodisperse silica beads (Microparticles GmbH, Berlin, Germany) of 2.06 \mu m or 4.39 \mu m diameter (d) with a standard deviation of ±0.05 \mu m were added to a concentration of about one bead per mm\(^3\). The beads have a positive acoustic contrast factor \( \Phi > 0 \), leading to stable force equilibrium at the pressure nodes of the standing wave field, as shown in Fig. 1a. The ends of the channel were sealed with silicone oil (VWR, France) to avoid water-evaporation during the experiment, which could lead to a change of acoustic properties over time. After trapping a particle, it was manually moved to the middle of the channel length, width and height. Next, the automated measurement routine was started to position the microscope \( xy \)- and \( z \)-stages, to record data, and to control the acoustic oscillations. The standard measurement plane of about 1 x 1 \text{mm}^2 was defined by a grid of 8 x 41 points (x- and y-direction, respectively). The spacing in the y-direction was with 25 \mu m higher than the 150 \mu m in the x-direction. For the experiments that included the acquisition of multiple measurement planes at different heights, the objective piezo z-scanner was moved in steps of 20 \mu m. 4 different height planes were measured, thus covering a total height of 60 \mu m. Experiments at the different frequencies were done by performing a linear frequency sweep between 3 and 4 MHz. The sweep time was set to 0.1 ms per 1 Hz to be much slower than the characteristic time constant of the optical potential \( \tau_{opt} = \frac{1}{\tau_{pp}^2} d^2 = 2.36 \mu s \) for a 4.39 \mu m silica particle, where \( \rho_p \) is the particle density and \( \eta_f \) is the dynamic fluid viscosity. Each measurement point included 10 seconds of recording the QPD 3D signals (at 1250 kHz) for the trap calibration, before the acoustic oscillation was switched on to record the particle displacement (at 1 kHz) by ultrasound. The repeated trap calibrations monitor the quality of the measured data and were used to eliminate experiments that were affected by a non-constant trap stiffness (sometimes caused by collected multiple beads or dirt in the optical trap). After working through the pre-defined sequence of measurement points, all the collected data of the bead calibration, its displacement under ultrasonic exposure, the acoustic frequencies, and the spatial bead position within the flow cell, were saved for further analysis. After converting the QPD signals to 3D force signals (using the calibration data), a gliding averaging filter with a window size of 160 data points was applied to reduce fluctuations, due to Brownian particle motion. The data was carefully checked for aliasing.

2.4 Numerical modeling

The finite element model of the device was set up in COMSOL Multiphysics (Version 5.1, Stockholm, Sweden). The complexity of the model and the scale difference between the wavelength and the total device size require a large amount of mesh elements. Hence it was impossible to perform the simulation in 3D and a 2D model was implemented. The chip is composed of multiple layers shown in Fig. 1c. The bottom layer consists of silica glass (density \( \rho = 2240 \left( \frac{kg}{m^3} \right) \), Young’s modulus \( E = 6.0 \cdot 10^{10} (1+i/2420) [Pa] \), Poisson’s ratio \( \nu = 0.245 \)). The second layer consists of a silicon single crystal (density \( \rho = 2330 \left( \frac{kg}{m^3} \right) \), anisotropic elastic-
Fig. 1 Forces in the acoustic flow cell and their detection. a) Acoustic radiation forces (red and blue color) on the particle will result from the periodic pressure field (green). Local fluid flow vortices (in clockwise and anti-clockwise direction) are formed by Schlichting streaming and induce Stokes drag forces on the particles. These forces form the total acoustic force. The force wavelength $\lambda_F$ of the acoustic force field is two times smaller than the pressure wavelength $\lambda_P$ of the acoustic pressure field. b) Detection of forces on the trapped bead is achieved by splitting the detection light path. For each path, a plano-convex lens casts the light onto the QPD, which is located in the back focal plane (dotted line) of the condenser. Only for the $z$-detection the optics are chosen such that the detection spot overfilled the QPD. c) Schematic drawing of the clean-room fabricated device. The device consists of two glass slides bonded onto two silicon spacers (in grey) to create a channel with a height $z$ of 120 $\mu$m and a length $x$ of 76 mm. The width $y$ was either 2 or 4 mm. The outer dimensions of 76x22 mm$^2$ were chosen to fit into a standard microscope sample holder.
ity matrix $D$). The channel was filled with water (density $\rho = 997 \text{kg/m}^3$, speed of sound $c = 1497\text{m/s}$). The top is sealed with another layer of silica glass. The piezo (PZ26, material data from Ferroferm, Kvistgaard, Denmark) was glued to the topside of the device. The glue was modeled using a thin elastic layer of 0.005mm. We found that the presence of the immersion media and the microscope lens had an influence on the acoustic boundary conditions of the device. Because the area covered by immersion oil is much larger than the area of the measurement plane, the presence of the moving microscope lens only influences the acoustic boundary conditions in $z$-direction. On the top of the device a small oil droplet (density $\rho = 1600\text{kg/m}^3$, speed of sound $c = 1497\text{m/s}$) was introduced into the model. The microscope lens was modeled as an acoustic hard wall boundary condition on the top of the oil droplet. As the speed of sound in the oil droplet is not known, it was approximated using the viscosity boundary layers. The solid domains were modeled using the Helmholtz equation, hence ignoring the viscous boundary layers.

We aimed to minimize such artifacts by applying an improved, standardized production procedure of the devices (see methods). To visualize the nodal lines of the (0,22,0)-line mode formed at 3.82 MHz in the 4mm wide device, we used very high concentrations of 9.6μm copolymer beads (Duke Scientific Corporation, Palo Alto, CA, US) with a positive $\Phi$ for better visualization (Fig. 2a). As expected, the lines were formed in $x$-direction by a standing wave in $y$-direction.

Despite the careful manufacturing procedure, the nodal lines in Fig. 2a show variations in straightness and density along $x$, which indicates a non-ideal pressure field distribution in the fluid channel. After washing out the 9μm reporter beads with pure water, we set out to measure the actual 3D pressure distribution in this area by using a single 2.06μm probing bead held in the optical trap. The red-framed area in Figs 2a and 2b indicate the 1x1mm observation plane in the middle of the channel ($z=0$).

Figs 2c-e show the $F_x$, $F_y$, and $F_y$-forces in the $xy$-measurement plane in three separate plots. The most prominent force occurs, as expected, along the acoustic wave direction ($y$). Fig. 2d shows $F_y$ for the predefined observation area. $F_y$ is fairly constant along $x$ with an average deviation of 20fN. Along $y$, the forces are harmonically distributed with an averaged amplitude of 172fN and with a force wavelength $\lambda_y$ that is consistent with the formed particle lines shown in Fig. 2a. The fitted wavelength $\lambda_y$ of 180.6μm is in excellent agreement with the theoretical $\lambda_y$ of 181μm. The acoustic trapping potential of $F_y$ is shown in Fig. 3a. Under the assumption that $F_y$ only results from acoustic radiation forces, we converted the measured forces into an acoustic trapping potential. The measured force field in $y$ was spatially integrated into the acoustic energy potentials based on Gor’kovs theory. To obtain the effective acoustic trap stiffness along $y$, we first averaged all wells in $x$-direction, followed by an averaging of the repetitive wells in $y$-direction. The resulting average potential well (Fig. 3b) has a parabolic shape, which proves a constant trap stiffness for small particle displacements out of the equilibrium position. The parabolic fit shows an average acoustic spring stiffness of 2.6$\text{N/m}$ with a linear range of over 50μm out of equilibrium. For comparison, the optical trap has a much higher stiffness of over 1000$\text{N/m}$ in the $xy$-plane, but with a linear range of just 1μm. The optical trap therefore is very suitable for probing the acoustic trap.

The orthogonal force $F_x$ (Fig. 2c), shows an irregular pattern with local maximal forces between -162/N and 140/N. In our measurement area these forces are on average five times smaller than $F_y$. Transferring this observation into the large-scale multi

$$k^2 = \frac{\omega^2}{c^2} = \left(\frac{\pi n_x}{l}\right)^2 + \left(\frac{\pi n_y}{w}\right)^2 + \left(\frac{\pi n_z}{h}\right)^2$$

Where $\omega$ denotes the acoustic frequency. The indices $n_x$, $n_y$, $n_z$ are the number of half-wavelengths in $x$, $y$ and $z$ directions of a channel with dimensions ($l \times w \times h$), respectively. The $n$-values also provide the number of nodal lines which are formed at resonance frequencies, which will hereafter be referenced as the $(n_x,n_y,n_z)$-line mode. In acoustofluidic devices, the nodal line formation of particles at a particular resonance frequency can be slightly distorted by scattering, and material and manufacturing uncertainties. This phenomenon has indeed been observed in several publications, e.g. 26,27.
The acoustic energy loss due to the clamping of the device on the microscope stage was taken into account by increasing the material damping (water, silica glass layers) in the 2D simulation. This procedure matches the resulting quality factor of the simulation with the measured quality factor $Q$ of the device at 3.82 MHz.

Although the simulation provides a realistic picture of the force distribution of $F_y$ and $F_z$, it fails to reproduce the 3D spatial local variations as observed in the measurements. Efforts to achieve better agreement would have to include all 3 dimensions but also sources of local energy dissipation (for example, due to the clamping of the device onto the microscope stage). This would rapidly exceed current computational possibilities for large devices and high frequencies. Thus, experimental testing remains essential to test the performance of acoustic flow cells and to serve as input for improving simulations. Our measurements show that the irregularities in the nodal lines with multi-particle experiments can be explained by local variations in the pressure field in all 3 dimensions. In the next sections, the individual influencing parameters will be investigated in more detail.

3.2 Force field evolution while changing frequencies

Different resonance frequencies will show different numbers of standing waves and nodal lines. With the optical trap based force probing technique, we have a unique tool to map the evolution of the pressure fields while changing the excitation frequency. Potentially, such information can be used to describe the particle behavior when particles are moved by continuous frequency changes inside acoustofluidic devices. To test this, we gradually increased the frequency from 3 to 4 MHz while measuring the forces on the probing bead in the optical trap. For these measurements we again mapped a 1 x 1 mm area like in Fig. 2b and averaged this into a single 1 mm line along the $y$-axis.

Fig. 4a shows the $y$-force of the measured force along this line on a 2.06 $\mu$m silica particle as a function of frequency inside a 2 mm wide channel device. The force field along $y$ shows several clear eigenfrequencies at 3.38 MHz $(0,9,0)$, 3.58 MHz $(0,10,0)$ and 3.82 MHz $(0,11,0)$. At these eigenfrequencies, we measured the periodicity of the force $F_y$ along $y$. When a constant force wavelength $\lambda_F$ was detected and the averaged force amplitudes were maximal, this was defined as an eigenfrequency. The amplitude distribution along the $y$-axis shows clear differences between eigenfrequencies. For example the $(0,11,0)$-line mode at 3.82 MHz is fairly regular in its amplitude but the amplitude distribution at 3.38 MHz $(0,9,0)$ shows a more pronounced variation along $y$. Between the eigenfrequencies, transition regions consisting of continuous lines of zero force (white) are visible, consistent with theoretical predictions from Trujillo et al. In the transition regions, the acoustic energy is about one order of mag-
ntitude smaller than at the eigenfrequencies. The highest forces are measured between the (0,9,0)- and (0,10,0)-line mode and they are an indication for the complexity of acoustofluidic devices. An analysis of the mode, similar to Fig. 2a, has shown that the line formation at 3.49 MHz is not homogeneously distributed along the y- and x-direction of the channel.

Fig. 4b shows that repeating the experiment with a different bead on a different day results in an almost indistinguishable periodic distribution of forces, which demonstrates the reproducibility of the experiment. However, the average acoustic force amplitudes for $F_z$ are slightly different (396\,fN for Fig. 4a and 348\,fN for Fig. 4b at $f = 3.82 MHz$), which might be caused by small differences in particle size or in mechanical boundary conditions, such as the mechanical clamping of the device on the microscope stage. Increasing the width of the channel should increase the number of possible node lines and may lead to different pressure amplitudes due to the different energy dissipation properties of the device. Fig. 4c shows the y-force of the measured total force on a 2.06\,µm silica inside a wider 4\,mm channel as a function of frequency. The eigenfrequencies of two different channels having $w$ and $2w$ width are directly related to each other for rigid boundary conditions. The eigenmode wave length of the smaller channel always fits into the larger one; the (0,n,0)-line mode of the w wide channel equals the (0,2n,0)-line mode of the 2w wide channel at the same frequency. The eigenfrequencies of the 2w width channel which have an odd number of modal lines, are not present in the w width channel. Indeed, in the 4mm channel several harmonic oscillations of the detected forces can be found, most prominently at 3.31 MHz (0,17,0) and 3.82 MHz (0,22,0). The (0,17,0)-line mode is not present in the 2mm channel but the (0,22,0)-line mode at 3.82 MHz is clearly visible as the (0,11,0)-line mode (Figs. 4a and 4b) and can be related to the (0,11,0)-line mode. Fig. 4e compares the amplitude plots of both channels at 3.82 MHz. The force amplitudes in the 2mm channel are about 2 times higher than in the 4mm channel, which shows that the coupling of the transducer oscillations into acoustic waves is less efficient for the wider channel. Fig. 4d shows the numerical simulation corresponding to the experiments with the 4mm channel in Fig. 4c. Six strong eigenfrequencies could be identified at 3.28 MHz, 3.37 MHz, 3.52 MHz, 3.58 MHz, 3.76 MHz, and 3.81 MHz. The latter agrees with the measured (0,22,0)-line mode at 3.82 MHz. As compared to the experimental observations, the simulated modes have slightly higher Q-factors; the eigenfrequencies bands are narrow and well defined. The predicted eigenmodes in the simulation appear at slightly lower frequencies as compared to their corresponding measured eigenmodes. This is due to small differences caused by uncertainties in the parameters. Similar to the simulation in Fig. 2f the amplitude distribution is very regular, which does not represent the spatial local variations as observed in the measurements. This discrepancy suggests the presence of dissipative elements that are not included in the simulation, which underlines the importance of experimental testing in the design of acoustofluidic devices.

### 3.3 Separating radiation pressure from streaming by using different particle sizes

To detect possible streaming, we focused on the eigenfrequency at 3.82 MHz (0,22,0)-line mode in the 4\,mm channel and compared the 3D force distribution for two differently sized particles. Because the acoustic radiation force is proportional to the volume ($\propto r^3$) of the particle and the acoustic streaming force only to its radius ($\propto r$), one should in principle be able to separate their contribution by using particles of different sizes. Fig. 5 shows the acoustic forces $F_x$, $F_y$, $F_z$ on particles of 2.06\,µm and 4.39\,µm in diameter. In all directions the forces on the 2.06\,µm particles are considerably smaller but their distributions are remarkably consistent, see also supplementary Fig. 1 for frequency sweeps with different particle sizes. To find the ratio between the force amplitudes we calculated the respective average peak amplitudes $\hat{F}_{2.06\mu m}$ and $\hat{F}_{4.39\mu m}$ (Fig. 5, right panels).

For the $F_z$-data, Fig. 5e shows that the shape of the force fields of the two beads are basically identical. The ratio between the forces was 8.1±0.06 (mean ± s.e.m.), which is much more than the size ratio of 2.13 for the two different particles and close to the volume ratio of 9.69. This indicates that acoustic radiation forces dominate the particle motion. Fig. 1a shows that both the radiation and streaming forces act in the same direction towards the node in the middle of the channel. The radiation force can be separated from the Stokes drag force by solving the following linear coupled equations

$$\hat{F}_{2.06\mu m} = C_{ar} \cdot d_{2.06\mu m}^2 + C_{st} \cdot d_{2.06\mu m}$$

$$\hat{F}_{4.39\mu m} = C_{ar} \cdot d_{4.39\mu m}^2 + C_{st} \cdot d_{4.39\mu m}$$

where $d$ is the particle diameter, $C_{ar}$ is the force coefficient for the Stokes drag forces and $C_{st}$ is the force coefficient of the acoustic radiation forces. This separation is possible because the properties of the acoustic field are the same for both experiments. The solution of Eq. 2 and 3 provides $\hat{F} = \hat{F}_{ar} + \hat{F}_{st}$: for the 4.39 \,µm particle the acoustic radiation force $\hat{F}_{ar}$ and Stokes drag force amplitude $\hat{F}_{st}$ are 1009.5\,fN and 61.5\,fN and for the 2.06 \,µm particle 104.2\,fN and 28.8\,fN. This results in an acoustic pressure amplitude of 942 kPa and an acoustic streaming velocity in $y$ of 1.49µm/s. The acoustic pressure was calculated by Yosioka’s theory for a 1D standing wave and by using the measured average force wavelength. The streaming velocity was calculated by Stokes drag theory.

For the $F_x$ data, Fig. 5f shows again an identical shape for the force fields but the ratio between both amplitudes has reduced to 6.4, which indicates a larger contribution from the streaming. If the plane of the measurements is not exactly in the centre of the cell then these streaming forces would push the particles at the acoustic radiation force equilibrium towards the bottom or cover glass (Fig. 1a). The contributions of the acoustic radiation force $\hat{F}_{ar}$ and stokes drag force $\hat{F}_{st}$ for the 4.39 \,µm particle are 138.1\,fN and 22.9\,fN, and for the 2.06 \,µm particle 14.25\,fN and 10.75\,fN. This results in an acoustic streaming velocity in $z$ of 0.6\,µm/s.

The measured $F_x$ forces along $y$, shown in Fig. 5d, are much smaller and have a force scaling factor of 3.37. This value is
close to the size ratio of 2.13 and indicates that acoustic streaming dominates \( F_z \) in these experiments. In conclusion, the forces along the direction of the acoustic wave are dominated by the acoustic radiation. Both orthogonal force components can for a significant part be attributed to acoustic streaming. This phenomenon is not limited to the selected frequency and Eq. 2 and 3 can be solved for each single measurement point individually by using \( F \) instant of \( \dot{F} \). The averaged \( F = F_{ax} + F_{ay} \) fields as a function of frequency are shown in the supplementary data, supplementary Figs. 2.

3.4 Forces throughout the volume of the acoustic flow cell

The aforementioned results for \( F \) were all measured at a single plane in the middle of the acoustic flow cell. In order to evaluate possible long-range effects of the channel top and bottom boundaries, but also to monitor qualitative streaming patterns in \( xz \) and \( yz \), we repeated the experiment at different heights (in \( z \)) to obtain all force vector components throughout the volume of the device. In a standard 4mm channel, \( F \) acting on a 2.06µm particle at a fixed frequency of 3.82 MHz was measured in a stack of four \( xy \) planes each consisting of 4 x 41 measurement points. For \( z \) we chose intervals of 20µm: (−30, −10, 10, 30µm) with respect to the middle of the channel. Measurement planes (in \( z \)) were chosen to be more than 10 times the particle diameter away from the channel walls (top and bottom) to ensure optimal performance of the optical trap\(^{21}\). Fig. 6 shows the spatial distribution of the \( F_{x}, F_{y}, \) and \( F_{z} \)-component in each of the planes. The most prominent signal, \( F_{z} \) displayed in Fig. 6b, shows the typical harmonic oscillation in the direction of wave propagation in the two most central measurement planes. The pattern in the upper and the lower plane is less pronounced, but a periodic pattern can still be recognized. Although the measurement planes were chosen to be far away from the top and bottom of the channel wall, \( F_{z} \) is less homogeneously distributed closer to the channel bottom and cover glass plate, with a 35% decrease of positive and negative amplitudes. This decrease can be partly attributed to the expected change in acoustic streaming direction as shown in Fig. 1a and the \( z \)-dependence of the pressure amplitude. \( F_{z} \) shown in Fig. 6a are much smaller with corresponding force amplitudes between \(-79 f N\) and \(83 f N\). Similar to Fig. 2b, the force distribution is irregular and does not show a clear dependency on the height of the plane. The \( F_{y} \) forces in Fig. 6c show in all planes a periodicity that follows that of \( F_{z} \). The force data shows that the force vectors approximately represent the expected streaming profile as shown in Fig. 1a., where the rotation direction is different between the upper and lower acoustic streaming vortex.

4 Conclusion

We combined an optical trap with an acoustofluidic device to measure the forces that are exerted by ultrasound on small particles. To achieve sub-pico Newton force resolution, decoupled QPD detectors were used to detect the \( xy \) and \( z \) displacements of the probe-bead. With this setup the full complexity of forces in 3D can be resolved, showing the limitations of 2D simulations. Additional strong modes exist, that are not provided by the simulations, while others do not show up in the experiments. The use of simple scaling laws allows separating the Stokes drag from the acoustic radiation forces. The measured \( F_{z} \) amplitudes scale with the 3rd power of the bead radius which confirms that these mainly originate from acoustic radiation. The \( F_{z} \), which has not been measured before in acoustofluidic devices, reached values of up to 20 % of \( F \) and could for a large part be attributed to streaming effects. The relatively large contribution of \( F_{x} \) and \( F_{z} \), perpendicular to the characteristic standing wave mode direction, show the complexity of force fields inside acoustofluidic devices and the need for a 3D measurement method to analyze them. Although the complete predicted streaming vortices have not been visualized yet in the current experiments, the method could be extended to sample the force field around a single node at higher spatial resolution. Apart from providing quantitative data for an improved modeling of acoustic regimes in standard channel geometries, this method allows also for testing how geometric factors, like near-wall or sharp-edge acoustics\(^2\), affect the acoustic forces.

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**Fig. 2** Forces involved in the formation of nodal lines. a) Photograph of the (0,22,0)-line mode in a 4 mm wide fluid channel, excited at 3.82 MHz with an excitation amplitude of 10 Vpp. The channel was filled with 9.6 µm copolymer particles (Φ > 0) for better visualization of the mode. The zoom shows the 1 x 1 mm measurement area with a five times higher particle concentration. b) Schematic drawing of the device with the measurement region indicated in red. c)-e.) Planar linear interpolation of the measured data points (black dots) for \( F_x \), \( F_y \), and \( F_z \), respectively representing the components of the total acoustic force on the particle. The \( F_x \) shows an uniform amplitude distribution with maxima and minima between 282 and -212 fN with an average periodicity of 0.362 mm (\( \lambda_F \)). The particles will accumulate at the pressure nodes where the force is zero (indicated with arrows). The \( F_y \) shows positive and negative forces at positions of pressure nodes in \( F_x \). \( F_z \)-forces are about \( \sim \frac{1}{8} \) of the forces of \( F_x \). A regular pattern of similar wavelength but shifted by about \( \frac{1}{4} \) of \( \lambda_f \) can be seen. f)- g) show the simulated radiation forces in 2D. \( F_y \) has a uniform distribution as function of \( x \), while \( F_z \) also shows some variation in direction of \( y \).
Fig. 4 Force fields on a 2.06\(\mu\)m particle at different frequencies for a 2\(\text{mm}\) (a,b) and 4\(\text{mm}\) channel (c,d). a) Increasing the frequency in a 2\(\text{mm}\) wide channel shows clear eigenfrequencies at 3.38\(\text{MHz}\); (0,9,0), 3.58\(\text{MHz}\); (0,10,0) and 3.82\(\text{MHz}\); (0,11,0). Only \(F_y\) is shown here. b) The robustness of the measurement method is demonstrated by obtaining near-identical results in an independent experiment. c) Experiments in a wider 4\(\text{mm}\) channel show an increase in the number of possible eigenfrequencies d) COMSOL Simulation of a 4\(\text{mm}\) channel shows strong eigenfrequencies at 3.28\(\text{MHz}\); 3.37\(\text{MHz}\); 3.52\(\text{MHz}\); 3.58\(\text{MHz}\); 3.76\(\text{MHz}\); and 3.81\(\text{MHz}\). The comparison of the predicted and measured acoustic quality factors \(Q\) of the single modes are in a good agreement. e) A comparison between the forces \(F_y\) in the 4\(\text{mm}\) and 2\(\text{mm}\) wide channel at 3.82\(\text{MHz}\). The force amplitudes of the 2\(\text{mm}\) channel are on average about 2 times higher than in the 4\(\text{mm}\) channel.
Fig. 5 Forces on small and large beads in a 4mm channel at 3.82MHz. The left graphs show the total forces on the 2.06µm (dark blue line) and the 4.39µm (light blue line) particles. a) $F_x$, b) $F_y$, and c) $F_z$; The right graphs show the force amplitudes comparison between the two particle sizes, normalized by the respective averaged peak amplitudes $\tilde{F}$ for both particle sizes. d) $F_x/\tilde{F}$, e) $F_y/\tilde{F}$, and f) $F_z/\tilde{F}$. 

Amplitude Ratio: 3.37
Amplitude Ratio: 6.44
Amplitude Ratio: 8.10

Total force $F_x$ [fN]
Total force $F_y$ [fN]
Total force $F_z$ [fN]
**Fig. 6** 3D Force distribution through the whole volume of the flow cell. The measurement region as shown in Fig. 2b was recorded at 4 different heights at 20 μm intervals in the flow cell a) $F_x$ shows no clearly defined periodic pattern, the pattern is different for each tested plane and points mainly in positive direction. b) $F_y$ shows a very regular periodicity in the center planes which deteriorates in the planes closer to the upper and lower channel boundary. The upper and lower planes were, however, still 30μm away from the boundaries. c) Like in Fig. 5, $F_z$ shows a periodic pattern that resembles that of $F_y$. The data is mostly consistent with the rotation direction change of the upper and lower acoustic streaming vortex (Fig.1a).
Supplementary Data

The supplementary data show detailed information about the results represented in Fig.5, where $F$ is decomposed in $F=F_\text{ar}+F_\text{st}$ by the use of the force scaling laws of two different sized particles.
Additional material to $F= F_{ar}+F_{st}$:

Averaged total force $F$ as a function of frequency for the two different particle sizes:

Supplementary Figure 1: Averaged total force $F$ as a function of frequency

a-f) show the measured force components $F_x$, $F_y$, and $F_z$ of $F$ as a function of frequency for the 2.06 $\mu$m and the 4.39 $\mu$m particle. The measured frequency range was 3-4MHz and the excitation amplitude was 10V_{pp}. 
Separation of acoustic radiation force and Stoke drag force due to acoustic streaming by the use of the force scaling laws:

Forces in y-direction:

Supplementary Figure 2: Averaged y-force component of \( F_{ar} \) and \( F_{at} \)

a) and b) show the measured y-force components as a function of frequency for the 2.06 \( \mu \)m and the 4.39 \( \mu \)m particle. The separation was done due to the different force scaling laws for the different sized particles. Supplementary figure 4.c and 4.d was point per point compared with each other by using the information that acoustic radiation force is proportional to the volume (\( \mathcal{V} \approx r^3 \)) of the particle and the acoustic streaming force only to its radius (\( \approx r \)). This comparison led to the separation of acoustic radiation and stokes drag force.