Three-dimensional matrixlike focusing of microparticles in flow through minichannel using acoustic standing waves: An experimental and modeling study

Claire Perfetti¹,⁎ and Carlo Saverio Iorio²,†
¹Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya, 466–8555 Japan
²Université Libre de Bruxelles, Service de Chimie-Physique CP 165/62, Av. Fr. Roosevelt 50, Brussels, 1050 Belgium
(Received 20 January 2016, Accepted for publication 8 April 2016)

Abstract: In this work, we demonstrate the possibility of focusing a stream of microparticles to generate a matrixlike distribution using bulk acoustic standing waves. To achieve this goal, an axial acoustic excitation was performed on a suspension of spherical quasi-monodisperse microparticles in a flow through minichannel with a square cross section of $2\text{mm} \times 2\text{mm}$. The pair of transducer elements was also used for stimulation at several frequencies corresponding to its theoretical eigenmodes. The generation of the matrixlike three-dimensional (3D) structures of focused particles was achieved by reflections of the acoustic radiation force associated with the square geometry. Particle positions were recorded by interferometric digital holographic microscopy, and the corresponding normalized distribution in the cross section was calculated for each experimental setting. The focusing efficiency was investigated through the variation of the acoustic energy density induced by the voltage applied to the piezo part and the injected flow rate. The acoustic field was numerically computed and compared with the experimental positions of particles in the cross section of the channel.

Keywords: Particle focusing, Acoustic standing wave, Minichannel, Acoustic radiation force

PACS number: 43.20.Ks, 43.25.Gf, 43.20.El [doi:10.1250/ast.37.221]

1. INTRODUCTION

Acoustic standing waves (ASWs) have been extensively studied as an efficient means of focusing particles or cells into pressure node or antinode positions for further processing such as separation [1], observation, agglutination [2–4] and concentration [5,6]. Most of these studies were carried out in rectangular channels and in a layered configuration, where a piezo actuator constitutes the bottom wall of the channel and an additional reflector is used to enhance the acoustic energy transmission. In this configuration, the height of the channel was chosen to match the $\lambda/2$ standing wavelength of the piezo transducer (PZT) fundamental mode to achieve a single pressure node located at half the channel height. Kuznetsova et al. investigated channel designs with height $h \leq \lambda/4$, which led, in combination with an appropriate reflector thickness, to a pressure node located at a different position from the midchannel [7]. In addition to the layered design with axial or transverse stimulation, Wiklund et al. worked on a device with multiple piezo parts orthogonally glued to the reflector: they achieved tree-dimensional (3D) handling of particles with a single focus in the middle of the channel [8,9]. Haake et al. used shear transducers to aggregate particles into lines and points [10,11]. Ravula et al. proposed 3D focusing of particles using a combination of ASW and dielectrophoresis that enabled the focusing of particles into one to ten lines [5]. ASWs have also been investigated thoroughly as an efficient means of particle manipulation, especially in the transverse actuation configuration. However, the lateral reflection of the acoustic wave and its ability to focus particles have been seldom reported, especially for a square cross-section channel.

In this study, the manipulation of particles by acoustophoresis was investigated in a square-crosssection glass minichannel ($2\text{mm} \times 2\text{mm}$), where ASWs were generated by two lead zirconate titanate PZTs glued on opposite lateral walls of the channel. This general configuration is depicted in Fig. 1. In this study, one transducer (PZT 1) is
excited electrically and generates an acoustic wave that propagates in the channel in the vertical direction through the glass wall. Owing to its identical features and symmetrical position, the second transducer (PZT 2) is mechanically excited and reflects the longitudinal wave. Moreover, we aim in this study to demonstrate effective particle manipulation under ASWs using affordable, standard, and commercial materials.

A solution of quasi-monodisperse particles of diameter ranging between 75 \( \mu \text{m} \) and 90 \( \mu \text{m} \) was injected through the channel. Particle migration under an acoustic field in the channel cross section was observed for several frequencies from 400 kHz to 1.2 MHz. As expected, no acoustic streaming effect was observed since the particles were too large [12,13]. The particle positions were recorded throughout the height of the channel by digital holographic microscopy (DHM) and the 3D coordinates were exported to MATLAB for further statistical analysis.

Two parameters were investigated and their relative impacts on focusing efficiency are reported. First, the variation of acoustic energy density induced by the voltage applied to the piezo part [12,14] was evaluated. Then, its relative sensitivity to the flow rate of the suspension was investigated.

2. THEORY

The radiation force potential derived by Gor’kov [15] for an arbitrary acoustic field on a spherical particle of radius \( r \) can be expressed as

\[
\langle U \rangle = \frac{4\pi r^3}{3} \left( \phi_1 \langle E_{\text{pot}} \rangle - \frac{3}{2} \phi_2 \langle E_{\text{kin}} \rangle \right),
\]

here \( E_{\text{pot}} \) is the time-averaged potential energy, \( E_{\text{kin}} \) is the time-averaged kinetic energy, and the material contrast factors \( \phi_1 \) and \( \phi_2 \) are defined as

\[
\phi_1 = 1 - \frac{\rho_m c_m^2}{\rho_p c_p^2},
\]

\[
\phi_2 = \frac{2(\rho_p - \rho_m)}{2\rho_p + \rho_m},
\]

\[
\langle E_{\text{pot}} \rangle = \frac{(p^2)}{2\rho_m c_m^2},
\]

\[
\langle E_{\text{kin}} \rangle = \frac{\rho_m (v^2)}{2},
\]

\[
\phi = \phi_1 + \frac{3}{2} \phi_2.
\]

In these expressions, \( \rho \) is the density of the fluid in the absence of perturbation, \( c \) is the sound speed, index \( m \) refers to the medium properties while index \( p \) refers to the particle properties, \( \langle p^2 \rangle \) is the time-averaged pressure, and \( \langle v^2 \rangle \) is the time-averaged velocity induced by the acoustic wave. Equation (1) shows that the force experienced by a particle is a function of the cube of its radius \( (r^3) \) and of the material contrast factors \( \phi_1 \) and \( \phi_2 \) between the particle and the fluid. When the acoustic force is integrated in the propagation direction, the acoustic contrast factor can be defined as a linear combination of the material contrast factors \( \phi_1 \) and \( \phi_2 \), and is commonly used to predict the particle behavior under ASWs. As a rule of thumb, the sign of the acoustic contrast factor determines if the suspended particles will be displaced toward the node or antinode pressure, a positive value corresponding to the former.

3. EXPERIMENTAL SETUP

The channel comprises a low-cost standard borosilicate glass tube tightly fixed to a Plexiglas/aluminum support (Fig. 2). The inner cross section of the channel is 2 mm \( \times \) 2 mm and the wall thickness is 0.4 mm (VitroCom). ASWs are generated by a PZ26 PZT (Ferroperm) (length = 25 mm, width = 25 mm, thickness = 5 mm) that is glued (Epotek 120E) on the piezo holder, where the electrical wires are also connected. A piezo component having the same characteristics is also glued symmetrically with respect to the channel to act as a reflector. The piezo parts are first glued to the Plexiglas supports with positive poling.
direction facing the glass channel. The electrical connection of both electrodes involved the welding of electrical wires inserted into the support holder in order to ensure strong robustness of the contact. Then the wires are glued to the positive and negative electrodes. In the case of the negative contact, the wire length is optimized to fit the length of the support. Finally the glass tube is inserted into the two extremities of the aluminum support, while the piezo parts mounted on their Plexiglas supports are simultaneously squeezed along the grooves of the bottom support until they reach the walls of the glass channel. The two piezo holders are then screwed into the support and cured. Finally, the fluidic connections (ibidi) are then implemented and waterproofed.

This experimental setup has high reliability and has been successfully used in many studies. This feature is of major importance when performing acoustophoresis experiments since ASWs are highly sensitive, and a slight modification of the system stiffness would result in a change in the resonant frequency.

3.1. Test Procedure

Tween surfactant was added to 80°C distilled water at a concentration of 0.1 v/v%. The solution was stirred and left to stand for 24 h. Then isobuoyant polyethylene particles (Cospheric) were added and the sample solution was degassed at 1.5 kPa for 20 min with a vacuum pump (N816.3KN.18, KNF Lab). Before each test, the fluidic setup, i.e., the capillary, channel, and tubing, were cleaned with a rinse solution of 70% isopropanol, injected via two independent pumping systems at a flow rate of 5 µl/min. Then distilled water was flushed at a flow rate of 50 µl/min for 10 min before air drying. At the beginning of each new test, the channel was previously emptied and the sample solution was injected with a flow rate of 5 µl/min. This step-by-step filling procedure was selected to avoid air bubbles from being trapped in the channel. When the hydrodynamic balance was reached, based on the observation of the particle flow through the observation window, the piezo device was activated. Once the acoustic field was fully established, 20 s of 25 Hz frame acquisition was started, and 500 images were then recorded. Each set of experiments was repeated four times.

3.2. PZT Impedance Measurements

As described previously, the particle-focusing efficiency will depend on the amplitude of the ASWs and thus on the voltage applied to the electrode of the PZT. To quantify the energy that was actually transmitted by the PZT to the system, its acoustic admittance should be evaluated. The PZT impedance $Z$ exhibits a minimum at its resonance frequency, corresponding to a maximum of the admittance $Y$, where the conversion of the electrical energy into mechanical energy is maximum. At this frequency, it is possible to model the PZT with an equivalent electrical circuit. Away from this working point, the impedance of a PZT should be empirically determined to obtain meaningful results.

The complex impedance of the electrically stimulated PZT has been measured in the setup configuration using an HP49349A analyzer with an additional HP43961A Impedance Test Kit (see Table 1). The frequencies were selected to correspond to exact multiples of the fundamental resonance mode of the PZT (expected to be around 402 kHz). If the admittance measured at 1.2 MHz was only ten times less than that at 400 kHz, the measurement of the admittance showed a very low value at 800 kHz. This illustrates the highly nonlinear behaviour of the PZT transducer which may be a problem in its application to industrial particle manipulation.

The complex admittance $Y = \frac{1}{Z}$ was then calculated and its real part $\Re(Y)$ was used to calculate the energy transmitted to the system when a voltage $V_{el}$ is applied between the two PZT electrodes. The electrical power $P_{el}$ that is effectively transmitted to the structure is thus calculated as

$$P_{el} = \Re \left( \frac{V_{el}^* V_{el}}{Z^*} \right) = \Re(Y^*)|V_{el}|^2,$$

here $V_{el}^*$, $Z^*$, and $Y^*$ are the complex conjugates of $V_{el}$, $Z$ and $Y$ respectively. Comparison of the focusing efficiency in terms of the electrical power transmitted divided by the voltage applied is preferable as large differences in the PZT impedance would lead to misinterpretation of the amplitude of the acoustic force.

4. RESULTS

4.1. Data Analysis

The visualization and tracking of particles are generally performed using particle image velocimetry (PIV) as in Manneberg et al. [8,16] and Barnkob et al. [17]. The tracking of particle motion under ASWs using the astigmatism particle tracking velocity (APTV) technique was also reported by Muller et al. [18]. In this paper, the 3D positions of particles in the flow were calculated through the numerical reconstruction of holograms record-

| Table 1 | Impedance and admittance measured at the selected frequencies. |
|-----------------|------------------|------------------|
| Frequency $f$ (kHz) | Impedance $Z$ ($\Omega$) | Admittance $\Re(Y)$ (m$^2$ $\Omega^{-1}$) |
| 400 | $190 - j227$ | 2.2 |
| 800 | $0.5 - j185$ | 0.00001 |
| 1,200 | $1.6 - j101$ | 0.2 |
ed by DHM. This technique is particularly suited to high-flowthrough applications since all the information in the imaged volume is included in a single hologram [19–21]. The working principle is described in [22] and the processing carried out for the automated generation of a complete coordinate set \((x, y, z)\) of all the particles has been previously used and explained in [23]. The recorded focus plane in the experiments was manually set to the half of the channel height so that the greatest number of particles could be optically focused in this area. Five-times magnification was used during these experiments, which led to a field of view of \(1,360\,\mu m \times 1,360\,\mu m\) and the capability of calculation with \(1\,\mu m\) accuracy. The \(z\)-coordinates of particles were in the range of \(-680\,\mu m\) to \(+680\,\mu m\) from the recorded plane.

### 4.2. Eigenmode Configuration

The observation of particle agglutination at specific locations in the channel was performed to confirm successful creation of pressure nodes characteristic of ASWs. The natural frequency of the 2-mm thick water medium at 25°C was evaluated as 376 kHz (with a sound speed of 1,507 m/s). The thickness-mode resonant frequency of the PZT is geometry-dependent and was evaluated by the manufacturer to occur around 402 kHz (in the poling direction) and around 60 kHz for the length and width resonant modes (orthogonal to the poling direction).

Figure 3 displays the positions of the particles in a cross section of the channel orthogonal to the flow direction. These coordinates were measured under the theoretical eigenmodes 0, 1, and 2 of the channel corresponding respectively to \(f_0 = 400\,kHz\), \(f_1 = 800\,kHz\), and \(f_2 = 1.2\,MHz\) and extracted using the data analysis process described previously.

Schematic diagrams of the expected maximum pressure distributions for fully established ASWs resulting from the excitation of the PZT in the thickness mode are plotted in the top part of Fig. 3. Owing to its specific layered configuration, ASWs generated by a PZT plate are expected to generate vertical nodal-line pressure nodes in the channel. Each diagram features the pressure distribution resulting from a specific frequency, while the intersection between the curves indicates the zero-amplitude pressure node.

In the bottom part of Fig. 3, the cumulative measured particle distribution in the cross section is shown by plotting the \((y, z)\) coordinates of the particles. The border of each figure corresponds to the inner border of the glass channel. Thus, the horizontal axis is the width of the channel while the vertical axis is the height of the channel. The central location of the cross section is represented by coordinates \((0, 0)\). Particles were focused around one to nine locations, numbered accordingly in the following sections on the parametric investigation.

As expected, particles were focused in the center of the channel cross section under the \(f_0\) resonant mode. For the \(f_1\) mode, instead of the expected alignment of particles into two streamlines, particles were observed to focus...
around four different locations. The average distance between two streamlines in the width direction of the channel was found to be 860 $\mu$m, which is slightly more than the calculated average distance between the two lines in the height direction of the channel (797 $\mu$m). The same 3D focusing effect was observed in the $f_2$ resonant mode, where particles were focused among nine different streamlines, creating a characteristic matrixlike structure. The average distance between the two streamlines in the width direction of the channel was found to be 585 $\mu$m, which is close to half the theoretical wavelength of the acoustic wave $\frac{\lambda}{2} = 628 \mu$m (using a sound speed for water of 1,507 m/s). The average distance between the two streamlines in the height direction of the channel was lower (498 $\mu$m), similarly to the $f_1$ mode.

### 4.3. Transmitted Power

As described previously, the particle-focusing efficiency will depend on the amplitude of the acoustic field. The mean standard deviations $\sigma$ of the above distributions are plotted in Fig. 4 for the focusing in the width direction (left column) and height direction (right column) of the cross section of the channel for the different eigenmodes. The horizontal axis is the transmitted power (mW/cm$^2$) and the vertical axis is the mean standard deviation $\sigma$ of the distribution of particles for each node location ($\mu$m). Nodes are numbered as in Fig. 3.

The general trend is that the focusing efficiency increases with the transmitted power up to an optimum value. Then, when the power is further increased, it leads to poorer focusing of particles. A tentative explanation of this observation is that multiple reflections of the ASWs in the square channel result in a two-dimensional (2D) node pressure. However, excess applied power can affect the PZT by modifying the electromechanical coupling. This nonlinear effect may be responsible for the decrease in the focusing efficiency. This assumption was investigated in

---

**Fig. 4** Graphs showing the evolution of the focusing efficiency with the transmitted power in the width direction (left) and height direction (right) of the cross section of the channel for the different eigenmodes. The horizontal axis is the transmitted power (mW/cm$^2$) and the vertical axis is the mean standard deviation $\sigma$ of the distribution of particles for each node location ($\mu$m). Nodes are numbered as in Fig. 3.
where a numerical model of the wave superposition effect was proposed. In the present study, the values of the transmitted power density were different for each eigenmode as they were calculated from the admittance measured at that specific frequency. As described previously, the aim of the present study was to investigate the focusing capability at the theoretical resonant frequency. Since the admittance decreased rapidly away from the resonant frequency, the transmitted power remained quite low, especially for higher eigenmodes. Nonetheless, the ability to focus particles under these conditions was successfully demonstrated.

4.4. Flow Rate

The impact of the velocity of the suspended particles on the focusing efficiency was also investigated (Fig. 5). Sets of experiments were carried out under the optimum transmitted power for each eigenmode, as described in the previous section, i.e., 563.2 mW/cm² for $f_0$, 196 mW/cm² for $f_1$, and 28.8 mW/cm² for $f_2$, while the suspension of particles was injected at different flow rates $Q$, from 0.05 ml/min to 2 ml/min.

The focusing effect seems to be more efficient for lower flow rates, as the exposure time to the acoustic field is higher. However, when the velocity of the particles is too low, a small sedimentation effect can be observed, decreasing the focusing efficiency in the height direction of the channel. In contrast, the higher flow rate of $Q = 2$ ml/min prevents particles from moving toward pressure nodes, owing to too short exposition to the ASW field and strong inertia. Under stationary conditions coupled with a high transmitted power, the agglomeration of particles into oscillating clusters was observed. The optimal flow rate of the suspension seems to be 0.5 ml/min for $f_0$ and $f_2$, and 0.2 ml/min for $f_1$.

5. FINITE-VOLUME MODELING

Numerical modeling was an important step for understanding and predicting the behavior of the experimental device and for helping interpret the results in the
The propagation of acoustic waves was calculated using a 2D unsteady laminar flow. The 2D model used, as displayed in Fig. 6, is a cross-section model orthogonal to the flow direction. This model has a total of 68,416 cells, their size ranging from 10 µm near the center to 2.5 µm close to the wall, where refinement is required to ensure suitable mesh deformation.

| Property                     | Symbol | Value   | Unit (SI) |
|------------------------------|--------|---------|-----------|
| Sound speed                  | c      | 1.507   | m/s       |
| Specific heat                | $C_p$  | 4.182   | J/(kg.K)  |
| Thermal conductivity         | $k$    | 0.6     | W/(m.K)   |
| Density                      | $\rho$ | User-defined | kg/m³    |

postprocessing phase. In this work, we used the commercial software Fluent™ together with the mesh generator Gambit™. Fluent™ belongs to the class of full Navier-Stokes (NS) equations solvers. It includes many standard discretization schemes and physical models that allow the implementation of application-specific terms in both the NS equations and the boundary conditions, strongly enhancing its numerical capability. Moreover, it can deal with very complex geometries. Simulations are performed on a 64-bits Intel® quad-core processor with 16Gb of RAM. Using this hardware configuration, the time required to achieve a meaningful simulation was roughly 1 day for a 2D unsteady laminar flow. The 2D model used, as displayed in Fig. 6, is a cross-section model orthogonal to the flow direction. This model has a total of 68,416 cells, their size ranging from 10 µm near the center to 2.5 µm close to the wall, where refinement is required to ensure suitable mesh deformation.

The modeling of acoustic wave generation and propagation is not part of the standard, built-in, physical models of Fluent™. Basically, acoustic wave generation was implemented by imposing a velocity condition at a moving boundary wall, which was coupled with a deforming mesh. The propagation of acoustic waves was calculated using a laminar model. The general form of the continuity equation for a compressible and unsteady flow can be written as

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \vec{v}) = 0.$$  \hfill (8)

The conservation of momentum is expressed as

$$\frac{\partial}{\partial t} (\rho \vec{v}) + \nabla \cdot (\rho \vec{v} \vec{v}) = -\nabla P.$$  \hfill (9)

### 5.1. Material Properties

The material properties used in the above model were based on the properties of liquid water liquid (see Table 2). However, the compressibility of the fluid was implemented using an equation of state of water, in which the density $\rho$ is expressed as a function of temperature $T$ and pressure $P$. The density was calculated using the equation of state developed by Chen in 1976 [25] for water for $0^\circ C \leq T \leq 100^\circ C$ and $0 \text{ Pa} \leq P \leq 10^8 \text{ Pa}$.

$$\rho = \frac{1}{v}, \quad v = \frac{v_0 P}{K_0 + AP + BP^2}$$  \hfill (10)

Here, $v_0$ and $v$ are, respectively, the specific volumes at pressures of 0 Pa and $P$, $K_0$ is the secant bulk modulus at $P = 0$ Pa, and $A$ and $B$ are temperature dependent parameters:

$$K_0 = -4.10^{-05} T^4 + 0.0122 T^3 - 2.2883 T^2 + 148.09 T + 19652,$$

$$v_0 = 4.10^{-10} T^4 - 7.10^{-08} T^3 + 8.10^{-06} T^2 - 6.10^{-05} T + 1.0002,$$

$$A = 9.10^{-10} T^4 - 8.10^{-07} T^3 + 0.0001 T^2 + 0.0005 T + 3.2614,$$

$$B = 4.10^{-12} T^4 - 1.10^{-09} T^3 + 9.10^{-08} T^2 - 6.10^{-06} T + 7.10^{-05}.$$  \hfill (11)

The sound speed is expressed as

$$c^2 = \left[ \frac{\partial P}{\partial \rho} \right]_S,$$  \hfill (12)

here the subscript $S$ indicates isentropicity. The sound speed was calculated from Eq. (10) in the model. However, its variation was negligible and setting a constant value did not significantly modify the results.

### 5.2. Moving Walls

The motion profile applied to the modeled piezo part and the rigid wall of the reflector was defined through a user-defined function as a sine function. The incident wave displacement was calculated by integrating the periodic boundary velocity $V^i$ over time. In the model, it is calculated as

$$V_{n,t}^i = A_0 \sin\left(\frac{2\pi t}{\tau N}\right).$$  \hfill (13)
here $n$ stands for the direction of propagation ($n = y$ or $z$), $t$ is time, $A_0$ is the amplitude of the signal, $T$ is the period of the signal corresponding to the half-wavelength condition for ASW generation, and $N$ is the number of pressure nodes expected.

The experimental observation of particle positions in the cross section suggests that ASWs were generated not only in the longitudinal direction of the PZT, but also in the orthogonal direction. Our explanation particularly relies on the square-cross section geometry of the channel, all the walls of which vibrate and reflect longitudinal waves. That is the reason why, instead of implementing a reflecting boundary condition on each wall but not the piezo device, the present model features a scattered wave similarly to the incident wave generated by the motion of the reflecting wall $V'$, This motion is also described by Eq. (13) with an additional parity condition associated with the expected number of pressure nodes $N$ in the channel: if $N$ is even, $V_{x, i} = V_{x, i}$, and if $N$ is odd, $V_{x, i} = -V_{x, i}$. In Fig. 6, the wave propagation from the piezo device is along the $y$ direction while the scattering wave from the orthogonal walls propagates in the $z$ direction.

The amplitude ratio between $A_x$ and $A_y$ (see Fig. 6) of the motion of these walls is investigated as the key parameter to model the asymmetry of the acoustic field resulting from a single stimulation in a square channel. The balance between $A_x$ and $A_y$ does not have a physical meaning except for $A_x = 0$ (ideal semi-infinite channel with no bottom and upper walls) and for $A_x = A_y$ (bottom and upper walls are submitted to exactly the same excitation). Intermediate values of the amplitude ratio indicate the vibration of the bottom and upper walls of the channel, leading to a 3D particle distribution in the channel.

### 5.3. Acoustic Potential

The static pressure and velocity field can only give a representation of the system at a specific time $t$, while the mean pressure and velocity remain zero owing to the exact superposition of waves under the ASW condition. However, the acoustic radiation force experienced by particles under an ASW depends on the second-order terms of the perturbation of the pressure and velocity. These terms are taken into account in the time-averaged pressure and velocity. The time-averaged values of the pressure and velocity are calculated in Fluent using the root mean square (RMS) of the pressure ($P_{\text{RMS}}$) and velocity ($V_{\text{RMS}}$) fields, which are defined as:

\[
\langle P^2 \rangle = \frac{1}{\tau_{\text{end}}} \int_0^{\tau_{\text{end}}} P(t)^2 \, dt = P_{\text{RMS}}^2,
\]

\[
\langle V^2 \rangle = \frac{1}{\tau_{\text{end}}} \int_0^{\tau_{\text{end}}} V(t)^2 \, dt = V_{\text{RMS}}^2.
\]

However, the contrast factors were calculated by Eq. (6) using $\rho_m = p_m^{(x,z)}(t)$ and $c_m = 1,507 \, \text{m/s}$ for the medium, the material properties of the suspended polyethylene particles in the experiments, $\rho_p = 998.2 \, \text{kg/m}^3$ and $c_p = 2,430 \, \text{m/s}$ for the particles. The acoustic potential was calculated in Fluent using a custom field function, where the time-averaged kinetic and potential energies were implemented using Eq. (1).

### 5.4. Numerical Comparison

The acoustic potential field was numerically computed (using $A_0 = 4 \, \text{cm/s}$) and the resulting pattern was compared with the experimental positions of particles. Figure 7 displays the superposition of the numerical solution and experimental observation. In this figure, particles are...
displayed as black dots. For clarity, a gray scale has been used ranging from white (where the acoustic potential field reaches its minimum) to black (highest values). From Eqs. (15) and (14), the node positions correspond to the minimum of the acoustic potential field and are thus the expected locations where particles are focused under ASWs.

\( A_y \) is the amplitude of the wall displacement induced by the piezoe device while \( A_z \) is the amplitude of the displacement of the walls orthogonal to the piezoe device due to the reflection of the acoustic wave as described in Fig. 6. The impact of the relative amplitude between these borders is investigated as a key factor to understand the pattern of particle positions in the cross section of the channel when an ASW is issued. Figure 7 displays the numerical pattern for different ratios of \( A_z \) to \( A_y \) for the case of a three-node ASW. The wavy experimental pattern (particle diameter range 75–90 \( \mu m \), \( P_{el} = 28.8 \, mW/cm^2 \), \( Q = 1 \, ml/min, f_z = 1.2 \, MHz \) best matches the simulation results for a numerical acoustic amplitude of \( A_z = \frac{1}{4} A_y \).

6. CONCLUSION

We experimentally demonstrated the focusing capability of an assembly including a fluidic square channel of 2 mm \( \times \) 2 mm cross section. Most previous studies on the acoustic manipulation of particles focused on achieving a perfect match between the resonant frequencies of the PZT and the medium as the admittance decreases rapidly outside a narrow bandwidth of 10 Hz order. However, this restrictive feature limits to its industrial application. In the present study, ASWs were generated with frequencies of multiples of the theoretical natural resonant frequency, thus demonstrating that particle manipulation using ASWs is not constrained by the need for a perfect matching between the transducer and the channel and may be effective in a greater range of frequencies.

For each resonant mode, a detailed statistical analysis was performed to find the optimal focusing conditions with respect to variation in the transmitted power and the sampling flow rate. This allowed us to conclude that in such devices, the transmitted power may be an important driving force, while the influence of the sampling flow rate is less marked, at least in the usual range of flow rates used in biological applications, provided that the particle sizes is not in the sub-micrometer scale.

Statistical analysis of the different resonant modes was presented along with the observed characteristic topology of the focused streams. Surprisingly, particles were distributed in a section orthogonal to the main flow axis in a matrixlike structure, sometimes collapsing into ribbonlike tracks. This present paper also demonstrates the ability to focus particles outside the exact resonant frequency, but in a frequency range based on only theoretical considerations (material and geometry). This result is expected to be of great interest when scaling up ASW devices for applications such as particle sorting.

REFERENCES

[1] H. Tsutsui and C.-M. Ho, “Cell separation by non-inertial force fields in microfluidic systems,” Mech. Res. Commun., 36, 92–103 (2009).
[2] M. Grundy, K. Moore and W. T. Coakley, “Increased sensitivity of diagnostic latex agglutination tests in an ultrasonic standing wave field,” J. Immunol. Methods, 176, 169–177 (1994).
[3] M. Grundy, W. Boles, W. Coakley and E. Benes, “Rapid agglutination testing in an ultrasonic standing wave,” J. Immunol. Methods, 165, 47–57 (1993).
[4] J. J. Hawkes and W. Coakley, “A continuous flow ultrasonic cell-filtering method,” Enzyme Microb. Technol., 19, 57–62 (1996).
[5] S. K. Ravula, D. W. Branch, C. D. James, R. J. Townsend, M. Hill, G. Kaduchak, M. Ward and I. Brener, “A microfluidic system combining acoustic and dielectrophoretic particle preconcentration and focusing,” Sens. Actuators B Chem., 130, 645–652 (2008).
[6] A. Neild, S. Oberti, A. Haake and J. Dual, “Finite element modeling of a microparticle manipulator,” Ultrasonics, 44 (Suppl.), 455–460 (2006).
[7] L. A. Kuznetsova and W. T. Coakley, “Microparticle concentration in short path length ultrasonic resonators: Roles of radiation pressure and acoustic streaming,” J. Acoust. Soc. Am., 116, 1956–1966 (2004).
[8] O. Manneberg, B. Vanherberghen, J. Svennebring, H. M. Hertz, B. Önfelt and M. Wiklund, “A three-dimensional ultrasonic caging for characterization of individual cells,” Appl. Phys. Lett., 93, 063901 (2008).
[9] B. Vanherberghen, O. Manneberg, A. Christakou, T. Frisk, M. Ohlin, H. M. Hertz, B. Önfelt and M. Wiklund, “Ultrasound-controlled cell aggregation in a multi-well chip,” Lab Chip, 10, 2727–2732 (2010).
[10] A. Haake, A. Neild, D.-H. Kim, J.-E. Ihm, Y. Sun, J. Dual and B.-K. Ju, “Manipulation of cells using an ultrasonic pressure field,” Ultrasound Med. Biol., 31, 857–864 (2005).
[11] A. Haake, A. Neild, G. Radziwill and J. Dual, “Positioning, displacement, and localization of cells using ultrasonic forces,” Biotechnol. Bioeng., 92, 8–14 (2005).
[12] R. Barnkop, P. Augustsson, T. Laurell and H. Bruus, “Measuring the local pressure amplitude in microchannel acoustophoresis,” Lab Chip, 10, 563–570 (2010).
[13] S. M. Hagsater, T. G. Jensen, H. Bruus and J. P. Kutter, “Acoustic resonances in microfluidic chips: Full-image micro-piv experiments and numerical simulations,” Lab Chip, 7, 1336–1344 (2007).
[14] R. Barnkop, I. Iranmanesh, M. Wiklund and H. Bruus, “Measuring acoustic energy density in microchannel acoustophoresis using a simple and rapid light-intensity method,” Lab Chip, 12, 2337–2344 (2012).
[15] L. P. Gor’kov, “On the forces acting on a small particle in an acoustic field in an ideal fluid,” Phys. Dokl., 6, 773–775 (1962).
[16] O. Manneberg, S. M. Hagsater, J. Svennebring, H. Hertz, J. Kutter, H. Bruus and M. Wiklund, “Spatial confinement of ultrasonic force fields in microfluidic channels,” Ultrasonics, 49, 112–119 (2009).
[17] R. Barnkop, P. Augustsson, T. Laurell and H. Bruus, “Acoustic radiation- and streaming-induced microparticle velocities
determined by microparticle image velocimetry in an ultrasound symmetry plane,” *Phys. Rev. E*, **86**, 056307 (2012).

[18] P. B. Muller, M. Rossi, A. G. Marín, R. Barnkob, P. Augustsson, T. Laurell, C. J. Kühler and H. Bruus, “Ultrasound-induced acoustophoretic motion of microparticles in three dimensions,” *Phys. Rev. E*, **88**, 023006 (2013).

[19] T. Kreis, “Digital holographic interference-phase measurement using the Fourier-transform method,” *J. Opt. Soc. Am. A*, **3**, 847–855 (1986).

[20] M. Takeda, H. Ina and S. Kobayashi, “Fourier-transform method of fringe-pattern analysis for computer-based topography and interferometry,” *J. Opt. Soc. Am.*, **72**, 156–160 (1982).

[21] F. Dubois, N. Callens, C. Yourassowsky, M. Hoyos, P. Kurowsky and O. Monnom, “Digital holographic microscopy with reduced spatial coherence for three-dimensional particle flows analysis,” *Appl. Opt.*, **45**, 864–871 (2006).

[22] F. Dubois, M.-L. N. Requena, C. Minetti, O. Monnom and E. Iorra, “Partial spatial coherence effects in digital holographic microscopy with a laser source,” *Appl. Opt.*, **43**, 1131–1139 (2004).

[23] C. Perfetti, C. Iorio, A. El Mallahi and F. Dubois, “Quantitative analysis of 3D hydrodynamic focusing of microparticles by digital holographic microscopy,” *Exp. Fluids*, **55**, 1–12 (2014).

[24] C. Iorio and C. Perfetti, “Acoustic potential generation under acoustic standing waves modeling using cfd software,” *Fluid Dyn. Mater. Process.*, **11**, 27–78 (2015).

[25] C.-T. Chen, R. A. Fine and F. J. Millero, “The equation of state of pure water determined from sound speeds,” *J. Chem. Phys.*, **66**, 2142–2144 (1977).

Claire Perfetti received a Ph.D. in Engineering from University of Brussels, Belgium, in 2014. She is currently working as a JSPS postdoc fellow at Nagoya Institute of Technology.

Carlo Saverio Iorio received his Ph.D. in Engineering from University of Brussels, Belgium, in 2006. His research interests include fluid mechanics, heat transfer, and biomedical sensing.