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## Characterization and control of a broadband acoustic cavity: Application to cells spheroids manipulation.

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### Introduction

The acoustic energy density  $E_{ac}$  is a key parameter for the acoustophoretic devices and applications. It determines the magnitude of the acoustic radiation force (ARF) applied on small objects like particles or cells. The prior knowledge of the experimental  $E_{ac}$  over a large frequency spectrum gives fundamental information on the device and allow many different applications like moving the axial position of the acoustic levitation plane just by changing the acoustic frequency [1]. In this work we present an acoustofluidic chip working with bulk acoustic standing waves. The chip is designed to produce several acoustic nodes and to allow lateral optical access. It is characterized using Particle Image Velocimetry (PIV) measurements. It allows us to assemble cells spheroids while applying a constant acoustic force over a wide frequency, just by frequency tuning.

### Background

The acoustic cavity is modeled as a 1D multilayer system in order to estimate the acoustic field properties from the particles velocities. Three layers, composed by water, glass and air, are considered. By applying the matrix formulation given by [2] and implementing the boundary limits we can compute the theoretical acoustic velocity and pressure. A standing wave is generated in the system and leads to interactions with the small polystyrene particles about 15 $\mu$ m, suspended in the liquid. The Gorkov and Yosioka model can be used [3] as the Rayleigh condition is verified and the ARF can be calculated:

$$F_{rad} = 4\pi r_p^3 \beta k E_{ac} \sin(2kz + 2\phi)$$

with  $\beta$  the acoustic contrast factor,  $r_p$  the particle radius.

In order to measure the acoustic energy density, the particle motions are linked to the ARF. According to the Newton's second law, and neglecting the inertial effect and the buoyancy force, we can express the velocity of the particle moving toward the acoustic pressure node as:

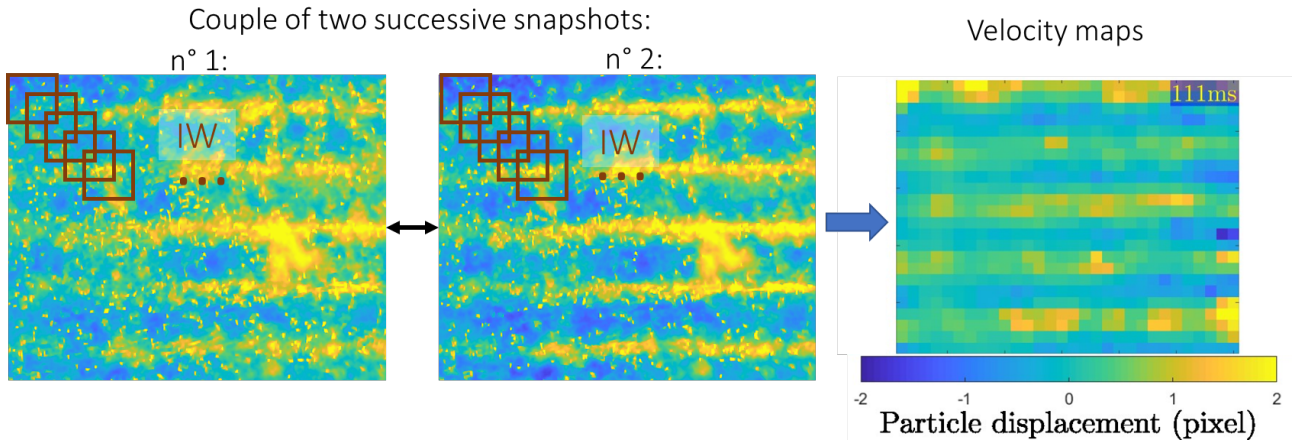
$$v_p(z) = \frac{1}{3\eta} 2\beta r_p^2 k E_{ac} \sin(2kz + 2\phi)$$

Measuring the velocity magnitude and knowing the particle diameter and physical properties, we access to the mean of the acoustic energy density.

### Experimental setup and PIV methods

The setup has been designed to be fully automated in order to carry out large parametric studies [4]. It allowed the measurement of the acoustic energy over a wide range of frequency with a small frequency step. For each acoustic frequency the particle suspension is mixed inside the acoustic cavity using a controlled syringe pump. Once the fluid is settled, a digital microscope and the ultrasound transducer are simultaneously triggered. The motions of the particles are then recorded until they reach the levitation plane.

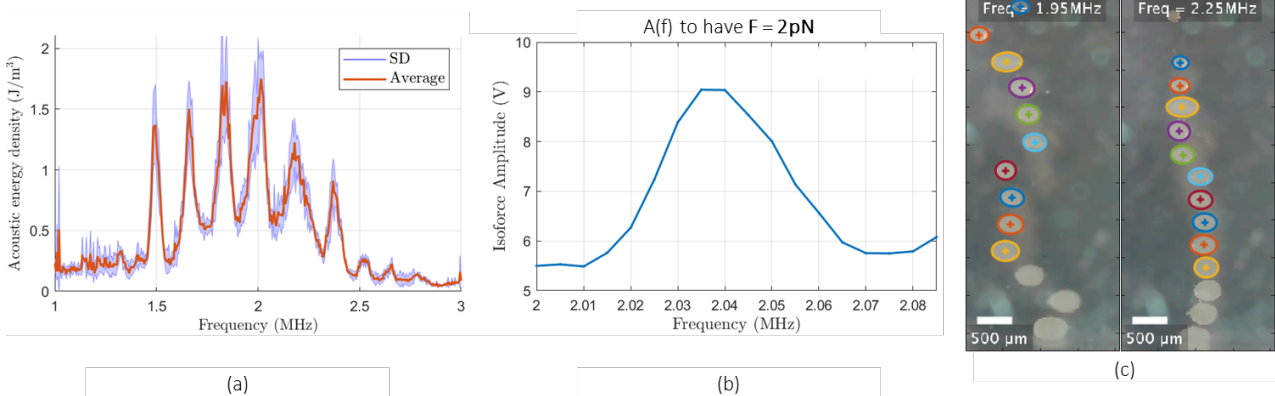
Using the successive snapshots, the PIV could be used to measure the polystyrene particles velocities. The velocity fields are computed using PIVlab [5], a 2D cross-correlation software. A velocity vector is computed on each interrogation windows (Fig. 1) leading to 2D velocity fields, which are averaged in the spanwise direction, allowing the computation of the maximum axial velocity of the particles.



**Figure 1:** Example of two snapshots acquired with the USB digital microscope. Interrogation windows (IW) are defined in order to compute 2D cross correlation. From global particle displacement, we obtained the velocity maps of the axial velocity of the particles moved by the ARF. This experiment was repeated many times to obtain robust characterization of the acoustic energy density.

### Acoustic energy measurements over a wide frequency range

We measured the evolution of the acoustic energy density for a given voltage (7Vpp) and for 2400 frequencies ranging from 1 to 3 MHz (Fig. 2a). Several resonance peaks can clearly be observed. It corresponds to the creation of new pressure nodes and to continuous shifting of the axial location of the levitation planes. The maxima of  $E_{ac}$  reaches  $1.75 J \cdot m^{-3}$ .



**Figure 2:** (a) Estimation of the acoustic energy density as a function of the frequency for a voltage of 7Vpp. (b) Calculation of the voltage amplitude to supply the transducer with a constant ARF on the particles. (c) Variation of the spheroid positions with frequency tuning. Two snapshots are showed but this manipulation was continuous.

### Isoforce manipulation of cell spheroids

The measurement of  $E_{ac}(F_{ac})$  provided the predefined pattern of the applied forces as a function of the frequency and the amplitudes of the generator signal. Using this knowledge, it was possible to change the amplitude of the signal to obtain a constant amplitude of the ARF while changing the frequency (Fig. 2b). The cell spheroids could then be moved closer to each other, and even assembled, just by tuning the acoustic frequency (Fig. 2c).

### Conclusion

One application of this work is the manipulation and merging of cell spheroids in acoustic levitation. After the self-organization of the cells in acoustic levitation, it was possible to bring them closer and closer just by varying the acoustic frequency. This manipulation allows the interaction between two spheroids in acoustic levitation.

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