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Selective optical manipulation of particles in acoustic levitation

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Abstract

Acoustic Radiation Force (ARF) is commonly used to create stable large-scale aggregates of particles in levitation (so-called "acoustic levitation") in a micro cavity. We show in the following work that this well-known and well-controlled aggregation process can be reversed without contact or external flow if the aggregated particles are enlightened with the proper optical wavelength. This coupled optics and acoustics effect has been observed with various kinds of particles and different optic wavelengths, showing high reproducibility. The phenomenon is studied using fluorescent micro-metric polystyrene particles without flow, and the effects of acoustic energy and illumination power have been quantitatively assessed. Since it is a tag free phenomenon, does not need high energies to happen and that it works with biological objects such as algae, red blood cells and bacteria, it may pave the way to a broad range of applications.

Introduction

Acoustic manipulation of micro-objects (particles, cells, bacteria, micro-bubbles, etc.) can be realized using ultrasonic standing waves in a fluidic or microfluidic resonator [1, 2]. Acoustic focusing of suspensions (or "acoustophoresis") is a complex phenomenon which has been the subject of many theoretical studies. It occurs in a micro-channel or a micro-cavity when the ultrasonic frequency f_{ac} , emitted into the cavity through a transmitter layer and reflected by the opposite wall (reflection layer), matches the resonance condition $\lambda_{ac} = 2h$, h being the height of the device (Fig. 1). In this case, particles such as plastic beads or living cells are drawn toward the acoustic pressure node created at mid-height of the cavity by the so-called Acoustic Radiation Force (ARF).

There are different theoretical models describing the ARF, based on more or less simplifying hypothesis [3, 4, 5]. King [3] was the first to propose computations on the forces on particles in a sound field based on several simplifying hypothesis. In particular, he considered the particles as rigid spheres. In the following, we will use the Yosioka model [4] which takes into account the compressibility of the spherical particles in the modeling. In this case, the primary Acoustic Radiation Force (ARF) F_{ac} responsible of particle acoustic levitation can be defined as:

$$\vec{F}_{ac} = \frac{\pi}{4} \langle E_{ac} \rangle k d_p^3 F_Y \sin(2k_{ac} z) \vec{e}_z \quad (1)$$

where $\langle - \rangle$ denotes time averaging, d_p is the particle diameter, $\langle E_{ac} \rangle$ is the time-averaged acoustic energy density inside the channel, $k = \frac{2\pi}{\lambda_{ac}} = \frac{2\pi f_{ac}}{c}$ is the wave number of the acoustic plane wave of frequency f_{ac} , F_Y is the contrast factor, a positive (for polystyrene particles in water) numerical constant, and z is the axial (or vertical) position of the particle, $z = 0$ being at the bottom of the channel and $z = h$ being at the top of the channel (Fig. 1). The z axis also corresponds to the wave propagation direction.

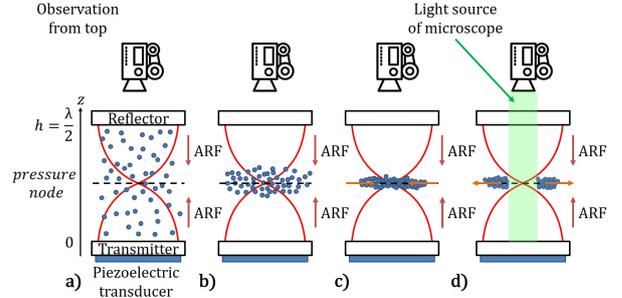


Figure 1: Principle of acoustic focusing of a suspension of particles in an micrometric acoustic resonator (a, b) and of the creation of an aggregate in acoustic levitation (c). Red lines denote the standing ultrasonic wave pressure profile. (d) The aggregate of fluorescent particles can then be broken up when, illuminated with a specific wavelength.

The acoustic contrast factor of a given particle of density ρ_p in a medium of density ρ_f is defined as:

$$F_Y = \frac{1 + \frac{2}{3} \left(1 - \frac{\rho_f}{\rho_p}\right)}{2 + \frac{\rho_f}{\rho_p}} - \frac{\rho_f c_f^2}{3 \rho_p c_p^2} \quad (2)$$

where c_p is the speed of sound in the particles material and c_f is the speed of sound in the fluid. In this investigation we are dealing with an ultrasonic standing wave with a frequency $f_{ac} \sim 2 \text{ MHz}$. The amplitude of the corresponding acoustic force typically ranges from 10^{-12} to 10^{-14} N (for instance, with polystyrene particles of $1 \mu\text{m}$ dispersed in our resonator filled with water, this force equals $2.8 \cdot 10^{-13}$ N).

Ultrasonic radiation forces are capable of levitating and aggregating in a fast and smooth manner a large number of particles or living cells in suspension in a cavity or channel. Once the particles have reached the nodal plane, the axial component of the force becomes null and the transverse component is no longer negligible. Indeed it has been shown that this component is about one hundred times weaker than the axial component and is then negligible during the focusing step [6]. Whitworth [7] derived the transverse component of the ARF for a radially symmetric acoustic wave in the nodal plane:

$$F_T = d_p^3 \frac{3(\rho_p - \rho_f)}{\rho_f + 2\rho_p} \nabla \langle E_{ac} \rangle \quad (3)$$

This transverse component depends directly on the radial gradient of the acoustic energy $\nabla \langle E_{ac} \rangle$ and is responsible for the aggregation of particles in the levitation plane toward the local maximum of acoustic energy.

Creation of large-scale aggregates under acoustic radiation force

As explained in the previous section, the acoustic radiation force is a simple and fast process to create large aggregates of particles in acoustic levitation. Once the ultrasonic wave is generated with the proper frequency into the cavity, the ARF forces the suspended particles to move toward the nodal plane, at mid-height of the cavity. Once the particles have reached the focusing plane, the transverse component of the ARF forces the particles to move toward the location of the maximum of acoustic energy. When the particles are close enough then the Bjerknes force keep the particles close together, making the aggregate more compact and stable. The creation of a large aggregate of particles is illustrated on Fig. 2 a) with $1.62 \mu\text{m}$ polystyrene beads. The focusing time T_{foc} can be very short (a few tenths of second), depending on the amplitude of the acoustic field. Once the aggregate is formed, it can be kept in acoustic levitation as long as needed and will remain stable, with the same spatial organization [8]. The aggregation process is efficient on particles as well as cells or active matter (bacteria). It can be used as a tool for creating an acoustic trap, especially for self-propelled organisms like bacteria that can be held in levitation in acoustic confinement [9].

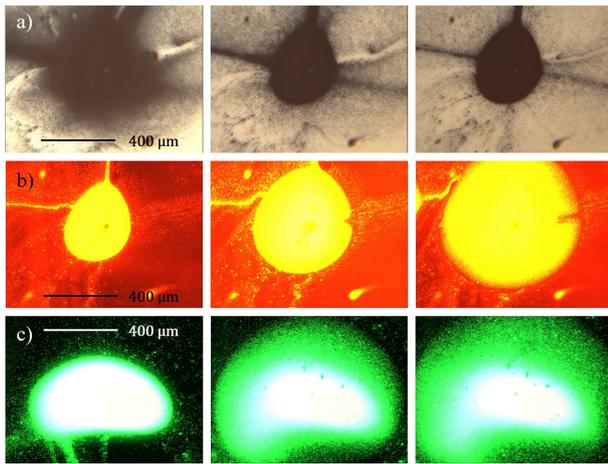


Figure 2: a) The three first pictures show the formation of a large stable aggregate of $1.62 \mu\text{m}$ particles. It can be kept stable as long as needed. b) When the same aggregate is illuminated with a green light ($\lambda_{light} = 545 \text{ nm}$) at a much higher intensity, the particles are quickly ejected from the illuminated region. c) We observe the same phenomenon with $1.75 \mu\text{m}$ particles fluorescing in green and illuminated with a blue ($\lambda_{light} = 488 \text{ nm}$) light at the same intensity. Time steps between frames is 200 ms .

Breakup of aggregates

As mentioned previously, once an aggregate of passive particles is formed using ARF, it remains stable and can be kept in levitation as long as needed. Without the use of an external flow or force, there is no way to break it up, apart from lowering enough (or turning off) the acoustics. In that case the aggregate either disintegrates in small aggregates and particles which all settle down, or the entire aggregate settles down, with particles still being stuck to each other by lubrication forces. That will be the case if the particles had time to interact strongly with each other.

There can be other behaviors with active matter, for instance when aggregating bacteria (active) or Janus particles (pseudo active). Indeed, when aggregated bacteria kept in levitation are

released from the confinement imposed by the acoustic field, the aggregate inner active pressure triggers an explosion-like breakup, radially ejecting bacteria confined in the acoustic field [9]. In the case of Janus particles, as demonstrated by Takatori et al. [10], the relaxation of an active crystal has been observed and described when the acoustic confinement is released.

What we shall report in the following is a new breakup process of aggregates of particles kept in acoustic levitation.

Breakup of aggregates of fluorescent particles by light

First observations

We first use fluorescent polystyrene particles of diameter $d_p = 1.62 \mu\text{m}$ which can be excited with green light and fluoresce in red light ($\lambda_{abs} = 545 \text{ nm}$ and $\lambda_{em} = 620 \text{ nm}$). Large aggregates of particles are created in acoustic levitation in the cylindrical resonator described on supplementary material figure at [URL will be inserted by AIP for SuppPub1.jpg] using $f_{ac} = 1.86 \text{ MHz}$ resonant frequency. As mentioned previously, the aggregates can be kept stable as long as needed when observed with a white light and at low power of illumination (The regular aggregation process can be seen in Fig. 2 a)).

Interestingly, when using a monochromatic ($\lambda = 545 \text{ nm}$) illumination, and gradually increasing the light intensity, the aggregation process is stopped. For even higher intensities, the aggregate starts to eject particles from its periphery. This ejection process goes on until disruption of the entire aggregate. If the light intensity is still increased, then this ejection process takes the form of an explosion, as can be seen on Fig. 2 b). One astonishing effect is that the particles escape the aggregate while remaining in the focal plane of the camera, i.e. in levitation. This result suggests that the primary radiation force (responsible for the acoustic focusing) is not affected, but that transversal and Bjerknes forces seems to be completely counterbalanced or screened by another force.

The disaggregation only occurs in acoustic levitation (no effect observed on beads that are on the bottom or top of the cavity) and when the particles are illuminated with the proper wavelength corresponding to the fluorescent particle absorption wavelength. In this case, for instance, the aggregate remains unchanged if illuminated with a blue light ($\lambda = 488 \text{ nm}$).

Another interesting point is that the effect is reversible: once the light intensity is lowered under a given threshold, then the standard acoustic aggregation process starts again with the same dynamics.

This phenomenon is highly reproducible and not limited to a given type of fluorescent bead. It has been tested with various fluorescent particles, all experiments leading to the same observations. Another example is shown Fig. 2 c), where an aggregate made from a solution of particles of diameter $d_p = 1.75 \mu\text{m}$ which can be excited with blue light and fluoresce in green light ($\lambda_{abs} = 450 \text{ nm}$ and $\lambda_{em} = 532 \text{ nm}$) is formed under an acoustic field of frequency $f_{ac} = 1.85 \text{ MHz}$. As soon as we illuminate this aggregate with a blue light ($\lambda = 488 \text{ nm}$ at 50 mW.mm^{-2}), the aggregate starts to eject particles from its periphery, as was observed before. The same expulsion phenomenon could be observed in a very reproducible way using these different fluorescences and with various diameters d_p , ranging from 0.883 to $5 \mu\text{m}$, when the aggregate was illuminated with the right wavelength.

Influence of the light intensity

As mentioned in the previous section, one can find a critical illumination intensity I_{crit} for which the acoustic radiation force

and the photo-acoustic force seem to balance. To further investigate the dependence of the effect on the amount of light injected in the aggregate, we did vary the light intensity for a given aggregate of $1.62 \mu\text{m}$ beads, at a fixed amplitude of the acoustic field. In the following the amplitude of the acoustic force is considered proportional to the acoustic energy inside the resonator (cf. Eq. 1). In this case, this energy is constant ($\langle E_{ac} \rangle = 106 \text{ J.m}^{-3}$).

We measure the ejection velocity v_{ej} for each illumination. The evolution of v_{ej} as a function of the illumination power P_{light} is plotted on 3 a). A linear evolution is found suggesting a direct proportionality of the ejection velocity with the injected illumination power: $v_{ej}(P_{light}) \propto P_{light}$.

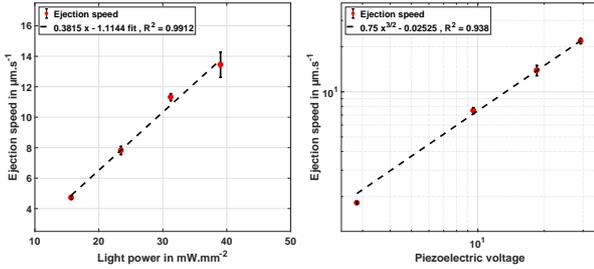


Figure 3: Evolution of the ejection velocity as a function of the illumination power (a) or of the amplitude of the ARF (b) for a given suspension of $r_p = 1.6 \mu\text{m}$ polymer beads.

Influence of the amplitude of the ARF

Similarly the influence of the acoustic field amplitude was investigated. We also found that the phenomenon was no longer observed when the acoustic field is turned off. This time, we did explore a range of ARF amplitudes ranging from 8 to 200 J.m^{-3} for a given illumination power (50 mW.mm^{-2}), all other parameters being kept constant

Using the same spatio-temporal analysis, we plot on 3 b) on logarithmic scale the evolution of v_{ej} as a function of the acoustic energy in J.m^{-3} for a given illumination power P_{light} . In this case, a linear evolution is found suggesting a scaling of the ejection velocity as a $3/4$ power law of the acoustic energy: $v_{ej}(\langle E_{ac} \rangle) \propto \langle E_{ac} \rangle^{3/4}$. Combining both results, and considering that both parameters are independent, one can define the following scaling law for the ejection velocity: $v_{ej}(P_{light}, \langle E_{ac} \rangle) \propto P_{light} \cdot \langle E_{ac} \rangle^{3/4}$.

Separation of a binary mixture

To demonstrate the ability of the photoacoustic effect to separate a binary mixture, we used a mixed solution of two colloidal particles: polystyrene particles of mean diameters $d_{p1} = 1.62 \mu\text{m}$ and $d_{p2} = 0.883 \mu\text{m}$, with absorption wavelengths $\lambda_1 = 532 \text{ nm}$ and $\lambda_2 = 450 \text{ nm}$ with an equal volume fraction of 0.025 %. First we created a large aggregate in acoustic levitation, following the same process as in the previous experiments (1.903 MHz and 200 J.m^{-3}) and using the same resonator. After a relatively short focusing time, the aggregate becomes stable in acoustic levitation and it contains an homogeneous mixture of both particles.

The upper row of Fig. 4 shows the aggregates in acoustic levitation but using different filters. Fig. 4 a) corresponds to an observation without filters. It shows a large aggregate in stable acoustic levitation. When using a green (Fig. 4 b) or red filter (Fig. 4 c) one can see that both types of particles are rather

homogeneously distributed over the entire aggregate. To our knowledge, it is impossible to separate such a mixture of particles once they are so well mixed.

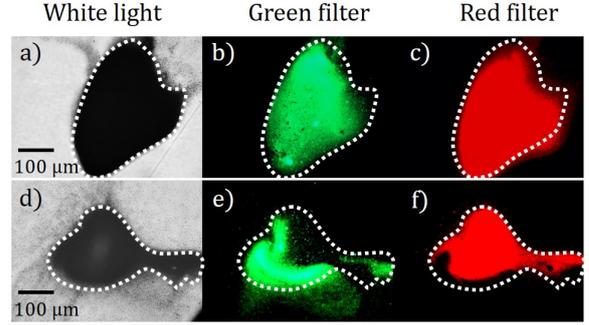


Figure 4: Separation of a binary mixture of $1.62 \mu\text{m}$ red fluorescent particles, and $0.883 \mu\text{m}$ green fluorescent particles. a), b) and c) pictures show the stable aggregate created by the acoustic force. The three pictures corresponds to observations without selective filter (a) and with respectively green (b) and red (c) filters. The pictures d), e) and f) show the aggregate after applying a strong illumination to the green fluorescent particles during 10 seconds and after all the ejected particles had time to focus again toward the aggregate. One can see that the new aggregate is no longer an homogeneous mixture of the two types of particles. It is now made of two clearly separate regions: one containing the red particles, the other one containing the green particles.

The aggregate has then been illuminated with a blue light ($\lambda_{light} = 488 \text{ nm}$) at a constant power of 30 mW.mm^{-2} during 10 seconds, then the illumination has been turned off. The resulting aggregate could then be observed once the particles aggregated under the influence of ARF. The result is shown on the lower part of Fig. 4. Fig. 4 d) shows the aggregate after a few seconds without any optical excitation. One can see that the aggregate has changed from its original shape. Fig. 4 e) and Fig. 4 f) shows the pictures of the aggregate when observed respectively with a green and red filter. We can clearly see that the aggregate no longer contains an homogenous mixture of both particles. The red fluorescent beads are now on one side of the aggregate while the green are now on the other side of the aggregate. This is a clear demonstration that the two types of particles have indeed been separated by the photoacoustofluidic effect. The separation was very fast (a few seconds) and efficient. The particles absorbing the illumination wavelength (the smaller ones in this case) have indeed been extracted from the aggregate before being attracted again toward the aggregate of larger particles, which did not breakup, because of the ARF.

Discussion

The previous experiments show a clear correlation between acoustics and illumination for the breakdown of aggregates. To evaluate this coupling, we studied the evolution of the ejection speed from the aggregates when varying the illumination power and amplitude of the ARF for a given suspension of particles ($d_p = 1.62 \mu\text{m}$). The result is shown on Fig. 5 as a phase diagram: we represent the initial ejection speed of particles from the aggregate as a function of illumination power and acoustic energy applied to the aggregate, i.e. a contour plot of $v_{ej}(P_{light}, \langle E_{ac} \rangle)$, the color bar being proportional to v_{ej} . As one can observe, the aggregate is locked in a stable state, provided that the illumination power is below 10 mW.mm^{-2} , or the acoustic energy of the ultrasound field is below 25 J.m^{-3} . Once this threshold is crossed (solid red line on the phase diagram,

corresponding to the Brownian motion at the experiment temperature, for these particles), then we have a more or less linear evolution of the ejection speed as light power or acoustic energy is increased. Interestingly, there seems to be an optimum for the ejection speed, as increasing the light power no longer increases the ejection velocity once the acoustic energy higher than 160 J.m^{-3} . One possible explanation for this behavior is that for these high acoustic energies, the acoustic streaming is no longer negligible, and may interact with the expulsion process, leading to some errors in the estimation of the initial ejection velocity.

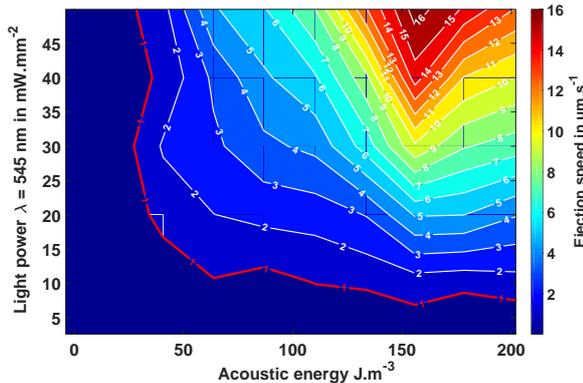


Figure 5: Determination of the phase diagram for an assembly of $1.62 \mu\text{m}$ red fluorescent particles. The ejection speed of particles from the aggregate are represented with a color scale, against the power of the light used on the aggregate and the squared voltage applied to the piezoelectric ceramic, representing the acoustic energy inside the resonator. The $1 \mu\text{m.s}^{-1}$ speed contour has been identified as the threshold between stable aggregate and aggregate breakup zones.

These results may suggest an amplified photo-acoustic effect on particles absorbing light in acoustic levitation. Indeed, photo-acoustic effect [11] takes place when a pulsed radiation is absorbed by an object: the thermal expansion of the material induced by the absorption of optical radiation causes dilatation and mechanical motion of the object then creating a sound wave propagating into the surrounding medium with the frequency imposed by the illumination source. Each particle thus becomes an acoustic source when properly illuminated. In our case the illumination is constant but the particles are already subjected to an acoustic force with a given pulsation frequency to create large aggregates in acoustic levitation. Both effects may lead to unexpected amplified photo-acoustic effect. The vibration of the particles induced by the ultrasounds may be amplified by the energy injected by the illumination. Further investigations are needed to clarify the origin of this phenomenon.

Conclusions

Choosing the proper geometric and acoustic parameters, it is possible to control the aggregation process of micro particles using our in-house ultrasonic resonators. Once the aggregates are formed, it is possible to keep them in acoustic levitation as long as desired. The only way to break the aggregates is to turn off the ultrasounds. Nevertheless we show that it is possible to destroy an aggregate of fluorescent particles just by illuminating it at the proper absorption wavelength. This phenomenon depends on both the acoustic force amplitude and the illumination power : the expulsion of the particles can only be observed in acoustic levitation, suggesting a strong coupling between acoustics and photonics effects. Above a given critical illumination power, the aggregate literally explodes. The expulsion of the

particles from the illuminated area can be very fast with an expulsion velocity which depends on the injected power (acoustic amplitude and illumination power). First experiments with dyed beads aggregates confirm that only the absorption properties are important in the process, not the fluorescence properties. Early observations also show that the phenomenon is affecting living cells such as red blood cells, opening the path to many applications of these findings. This phenomenon, which may be named *optoacoustophoresis* due to its dependence on both excitations, opens the path to many new manipulations and sorting processes of suspensions, combining both acoustic and optical properties of particles or cells. This new branch of acoustofluidics could be called *optoacoustofluidics*. The potential applications are numerous for both diagnosis or production, such as fine cell separation for analysis or rare cell tagging in continuous flow systems.

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