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## Study of the influence of microgravity on brain derived spheroids in acoustic levitation

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In a Bulk Acoustic Wave (BAW) resonator, spherical objects, such as particles, can be maintained in an equilibrium position, between the walls of a resonant cavity. In the so-called acoustic levitation plane, the gravity is counterbalanced by the Acoustic Radiation Force (ARF) created when the resonance condition is respected ( $h = \frac{1}{2}\lambda_{ac}$ , with  $h$  the height of the cavity and  $\lambda_{ac}$  the acoustic wavelength).

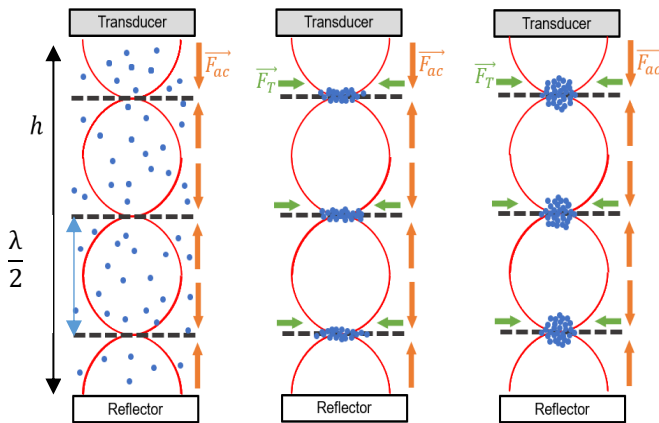


Fig. 1: Acoustic resonator applied to cells (blue points). We observe, thanks to the acoustic radiation force, the rapid formation of layers of cells in the acoustic nodes. After a few hours, the cell sheets turn spontaneously into spheroids.

The analytical expression commonly used to describe the ARF applied to a single spherical particle is obtained by assuming that the diameter  $d_p$  of the irradiated particle is very small compared to the acoustic wavelength ( $d_p \ll \lambda_{ac}$ ). This assumption is called "Rayleigh's approximation" or "Rayleigh's limit" [1,2]. It is therefore perfectly applicable to objects of microscopic sizes ( $d_p = 1$  to  $20 \mu m$ ) and an ultrasonic frequency of the order of a few MHz (i.e.  $\lambda_{ac}$  of the order of a few hundred  $\mu m$ ). In this context, particles or cells can be moved toward the acoustic levitation plane where they can form large aggregates.

We introduce a new scaffold-free approach to structure and cultivate organoids using multi-nodes

acoustic levitation. It can be used to create large aggregates of cells in a contact-less, label-free manner and with enhanced cell-cell interactions. We previously showed that human mesenchymal stem cells (hMSC) grown for 24 hours in acoustic levitation self-organized into spheroids and exhibited accelerated differentiation compared to classical 2D culture conditions [3]. These cultures also grow in a 3D environment where they can thrive, mimicking in vivo organs with more accuracy, which allows them to be used as model for organ growth or drug tests. Another advantage of acoustic levitation lies in the possibility to control and shape a dynamic mechanical force field, through ARF, around growing cell aggregates.

Moreover, this observation proves that another expression of the ARF should be derived for the case where  $d_p \approx \lambda_{ac}$ . In order to study more precisely the limits of this hypothesis, we carried out experiments in microgravity, during parabolic flights. In these experiments, the gravity no longer hinders the ARF and it becomes possible, even with small amplitudes ARF, to handle large and / or dense objects, which would otherwise sediment quickly in standard laboratory conditions on Earth.

A dedicated setup has been designed and built to run acoustic levitation experiments during parabolic flights. The setup should allow observation of particles or cells in acoustic levitation. A Zeiss Axiovert inverted microscope has been integrated in a Zargues box, together with a highly sensitive high-speed camera. A motorized moving plate and a stage incubator has also been integrated. All the parameters are controlled and monitored using computers and interfaces installed in a dedicated rack (Fig. 2).

Using this technology, we were able to observe the electrical activity of both 2D neuron layers and 3D neurons spheroids trapped in acoustic levitation, during the CNES parabolic flight campaign of

spring 2022. The analysis of this experiment will allow us to better understand the impact of gravity on neuronal activity in 3D as well as in 2D.

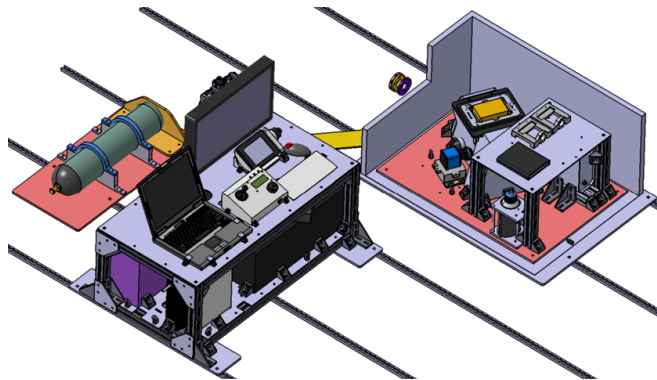


Fig. 2: Experimental setup used for the parabolic flights. On the left one can see the control rack, giving access to all parameters of the experiments. On the right, one can see a closed box in which an inverted microscope has been installed. The acoustofluidic cavity lies inside a stage incubator.

We will present in this talk the first experimental evidences showing that indeed ARF can move and levitate large objects far beyond the Rayleigh's limit. We will also show for the first-time that is indeed possible to monitor calcic activity of a neuronal spheroids in acoustic levitation. Preliminary results after this first campaign show that indeed the neuronal activity seems to be influenced by the variation of gravity, from hyper gravity (2g) to micro-gravity (0g).

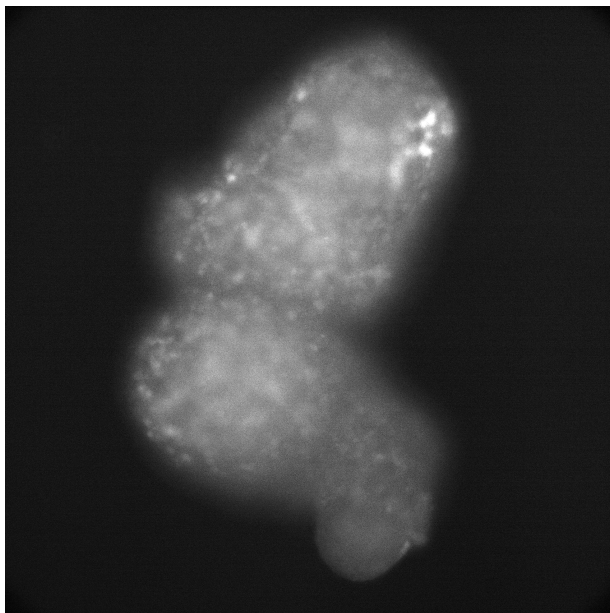


Fig. 3: Observation of two neuronal spheroids in acoustic levitation. Using fluorescence microscopy, it was possible to monitor calcic activity in the spheroids during the parabolic flights.

## References

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