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Acoustic intensity monitoring in a 2D cell culture under low intensity ultrasound stimulation

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Background, Motivation and Objective

Low Intensity Ultrasound (LIUS) stimulation is a therapeutic modality currently used clinically for bone regeneration. However, the **physical and biological phenomena** involved in the cellular process of **mechanotransduction** are still poorly understood. To gain insight into these multi-physic and multi-scale phenomena, **in vitro studies on 2D cell culture** dishes are essential, but the relevant interpretation of the results requires the evaluation of the intensity delivered to the cells. However, the **disturbing effects** related to the standing waves inside the culture medium resulting from reflections of the waves at the interfaces between the air, the culture medium and the dish, are generally disregarded. In this work, the objectives are the validation of an **anti-reflection cover**, adapted to culture dishes, allowing the elimination of standing waves, and the development of a **"twin" numerical modeling** allowing the **monitoring of the acoustic intensity** delivered to the cells.

Material & Methods

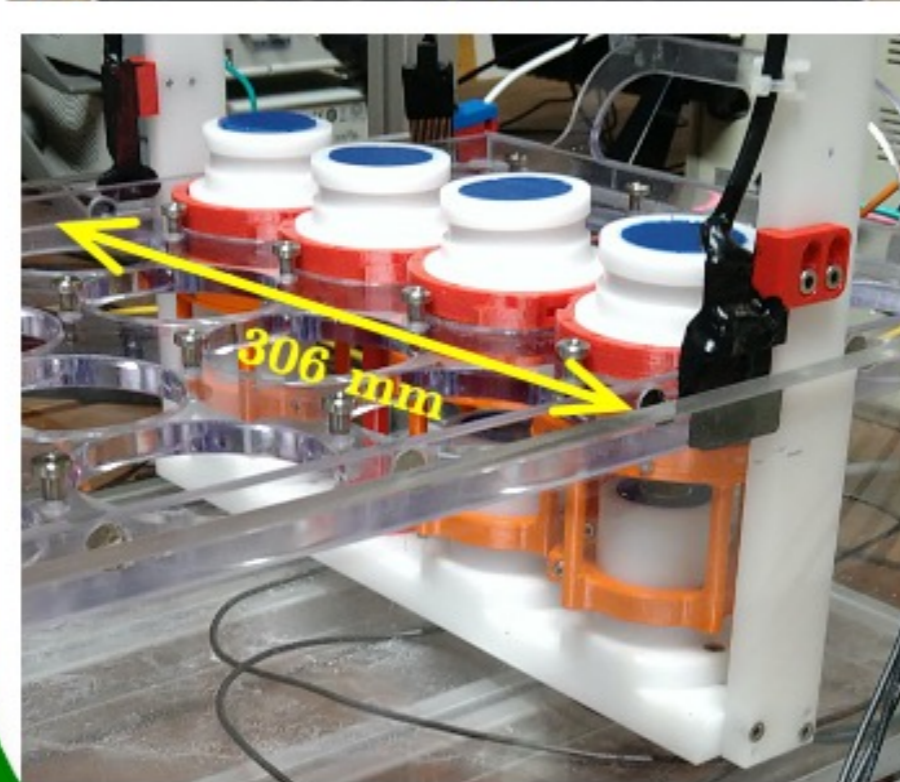
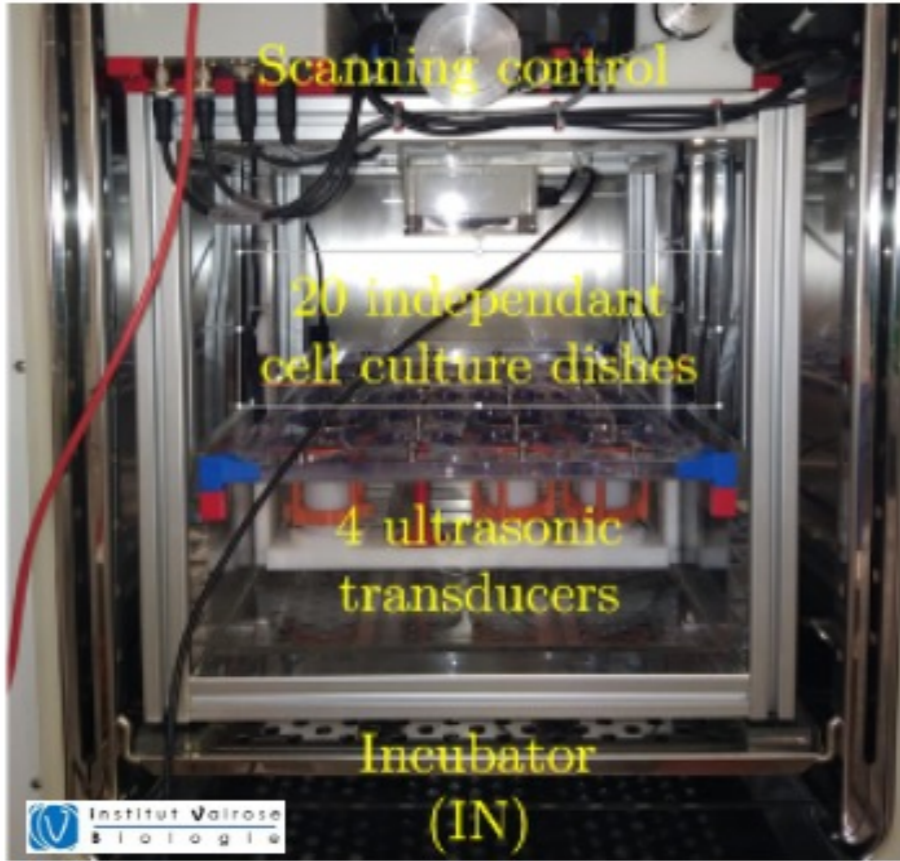
The cover, which is a **custom POM-tube** containing degassed water and a **thick absorbent foam**, was placed over a (cell-free) culture dish. Experiments were performed using a **1 MHz transducer** placed under the set. The intensity (I_{SPTA}) was evaluated using a needle hydrophone by scanning the xy-plane of the acoustic field under LIUS stimulation. **Three configurations were tested**: without (**case 1**) and with (**case 2**) the cover over the dish with a low water level inside, and without the cover but with a high column of water above (**case 3**). The **2D-axisymmetric "twin" modeling** was developed under COMSOL Multiphysics®, using the Pressure Acoustics module and Solid Mechanics module for the wave propagation in the fluid and solid parts, respectively.

Results/Discussion

The peak acoustic pressures obtained with the cover containing a low water level are equivalent to those that would be measured if the culture dish contained a high water column. The cover traps the transmitted waves, preventing standing waves from occurring in the culture medium, which is comparable to a column of water several centimeters high. The "twin" modeling is used to compare I_{SPTA} inside the culture dish. To conclude, through experimental and numerical modeling, we are able to predict and monitor the acoustic intensity in the 2D cell culture medium under LIUS stimulation.

2D cell culture

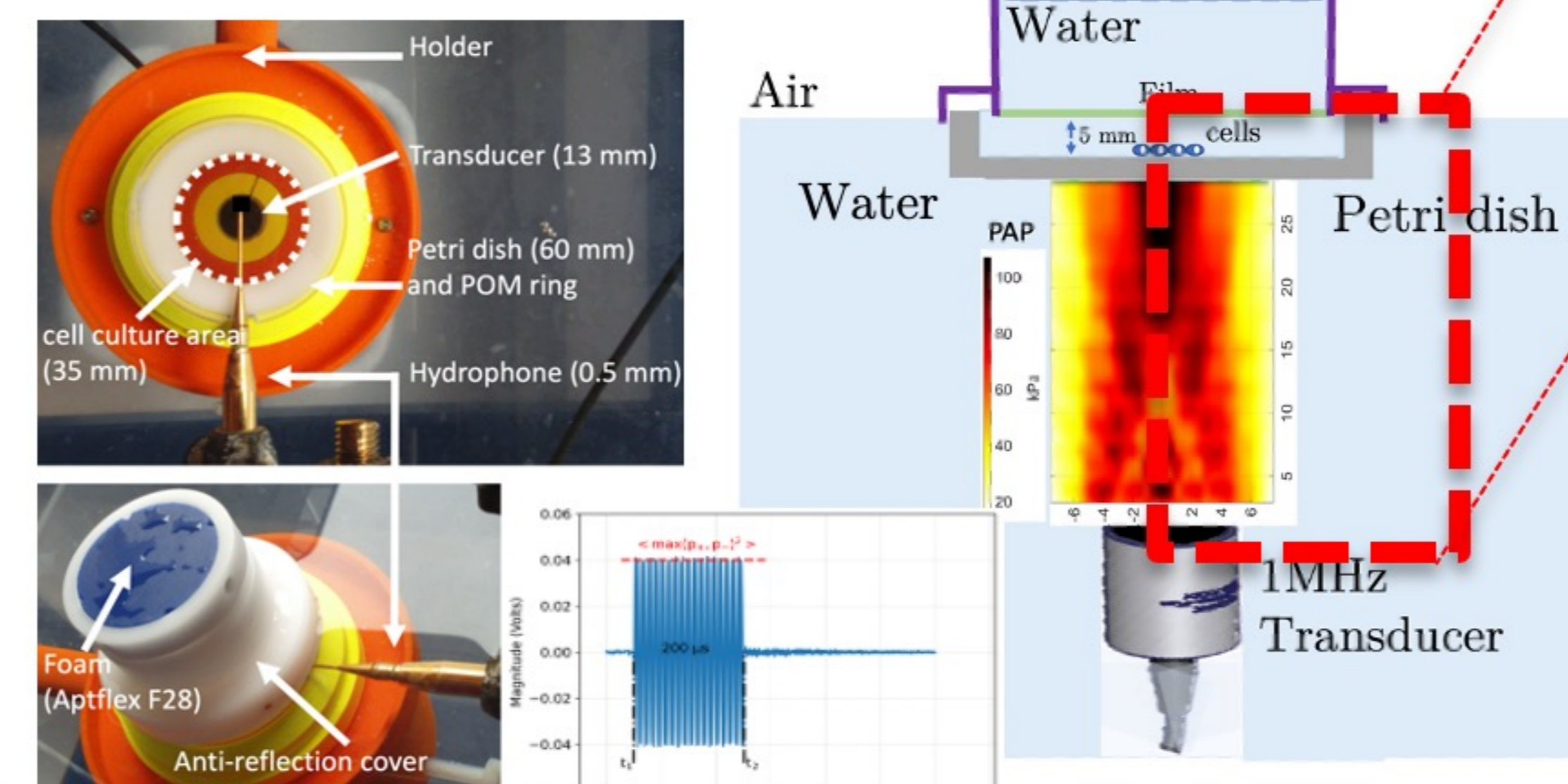
Serial LIUS-stimulator


 Co/ Vincent LONG (LMA, Marseille)
Julien HOULES (CPPM, Marseille)

Variable	Distance between	Value (mm)
z_f	the transducer ¹ and the hydrophone	28 (1 MHz),
D (high water level)	the transducer ¹ and the water-air interface	700
D (low water level)	the transducer ¹ and the water-air interface	46.3
h	the transducer ¹ and the Petri dish ²	25 (1 MHz),
a	the Petri dish ³ and the hydrophone	2.2

- (1) the active front face of the transducer.
- (2) the back wall and (3) the bottom of the Petri dish.

Anti-reflection cover


 200 μ s, 1MHz-burst, DC 20%

"Twin" modelling

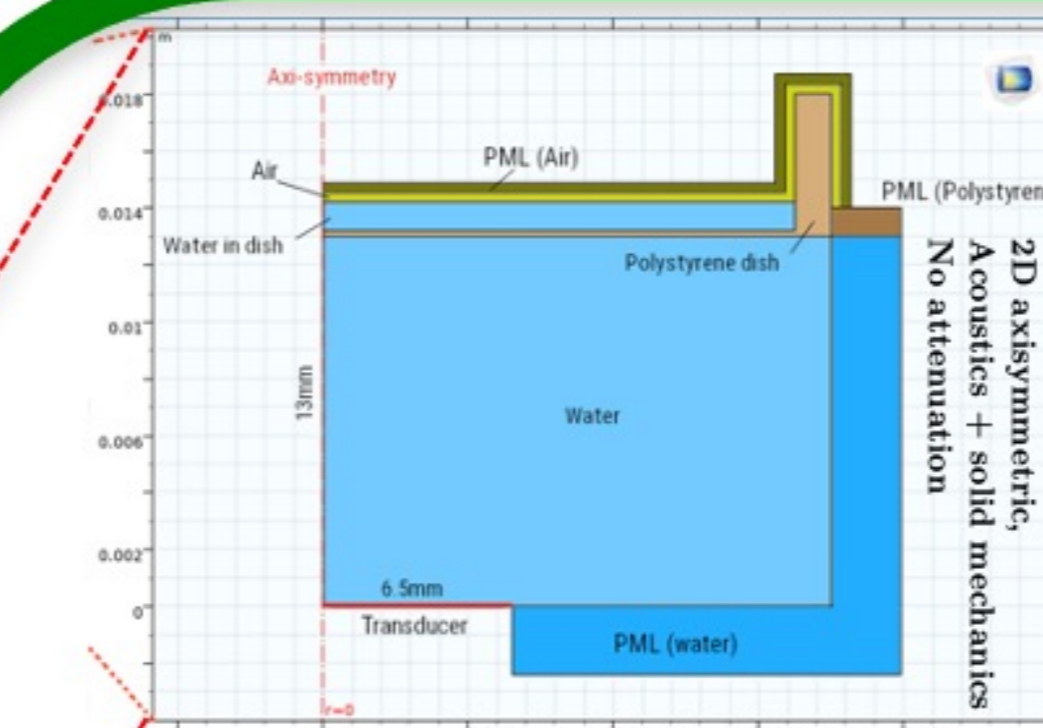
- Temporal Average Intensity: I_{TA}

$$I_{TA} = \frac{1}{T_{PRF}} \int_0^{T_{PRF}} \frac{p^2(t)}{\rho c} dt [mW/cm^2]$$

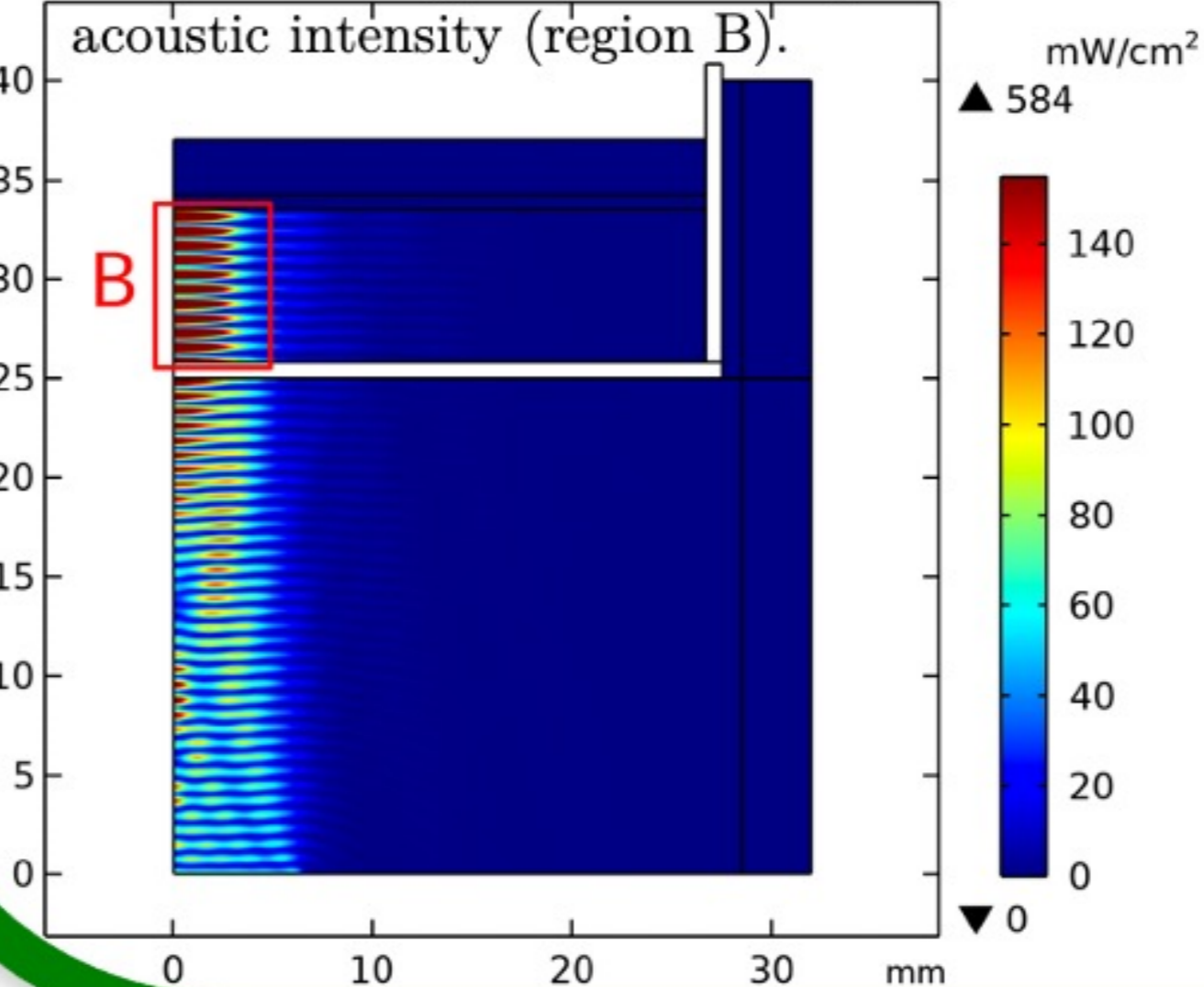
- Spatial Average Temporal Average Intensity: I_{SATA}

$$I_{SATA} = \frac{1}{S} \int_S I_{TA} dS [mW/cm^2]$$

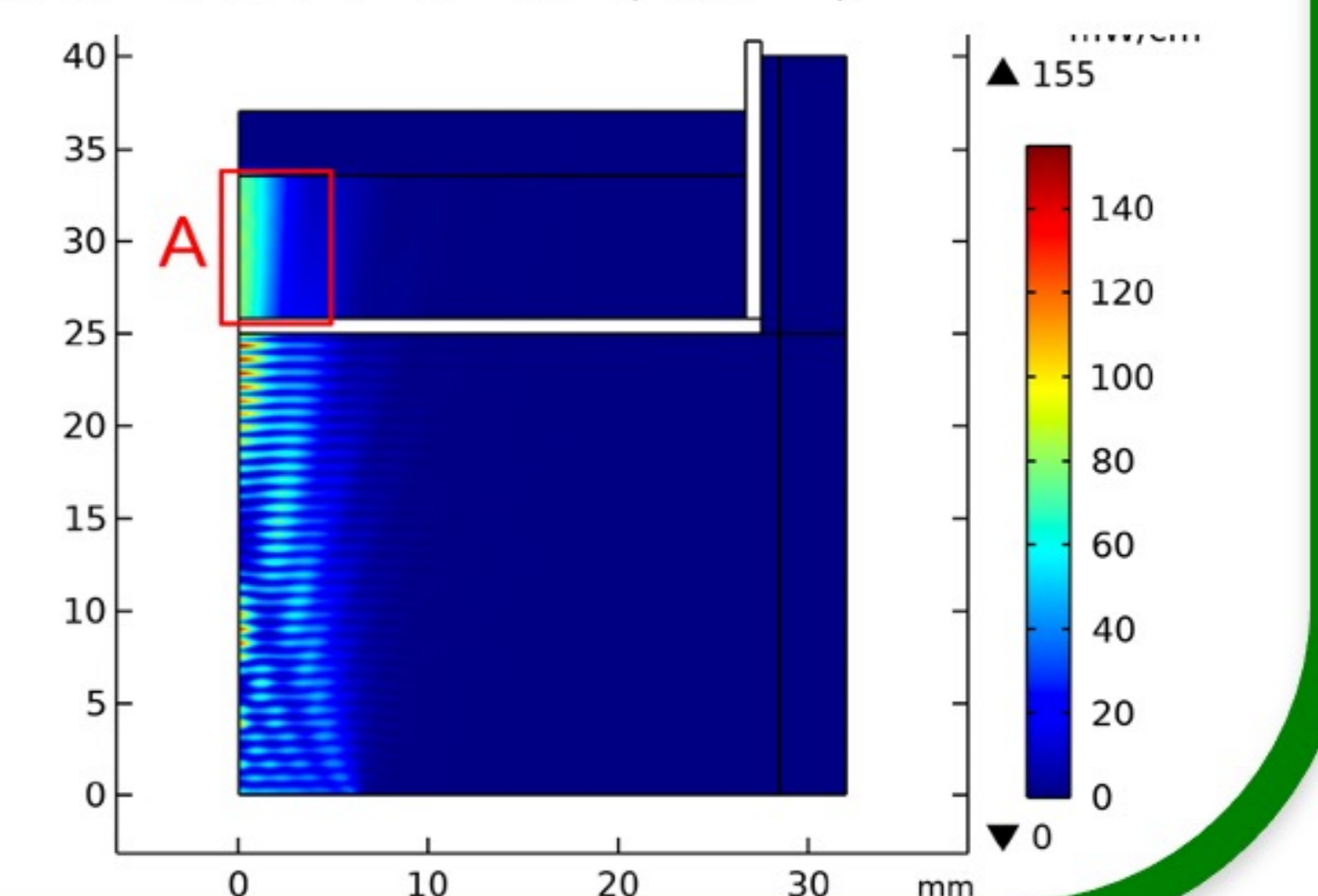
$$\forall I_{TA} : I_{TA} \geq 0.25 (I_{TA})_{max}$$



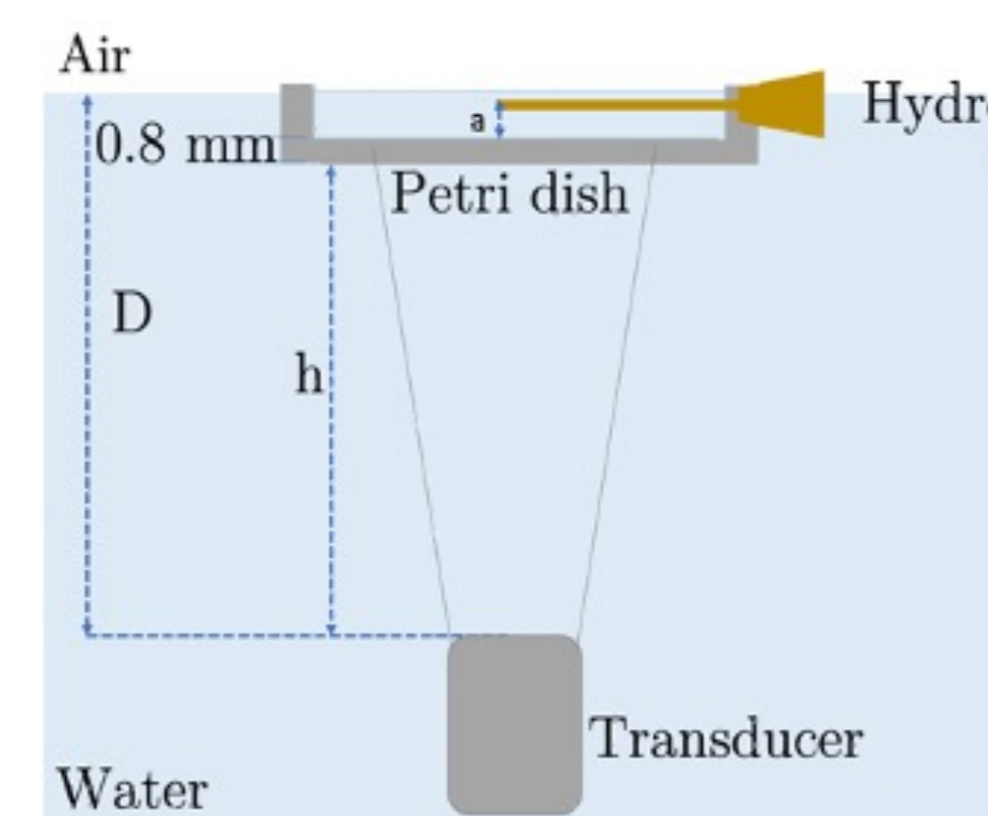
The **air PML (no absorbing device)** is applied on the upper border of the culture medium. Multiple reflection of the waves leads to formation of the standing waves and significant increase in acoustic intensity (region B).



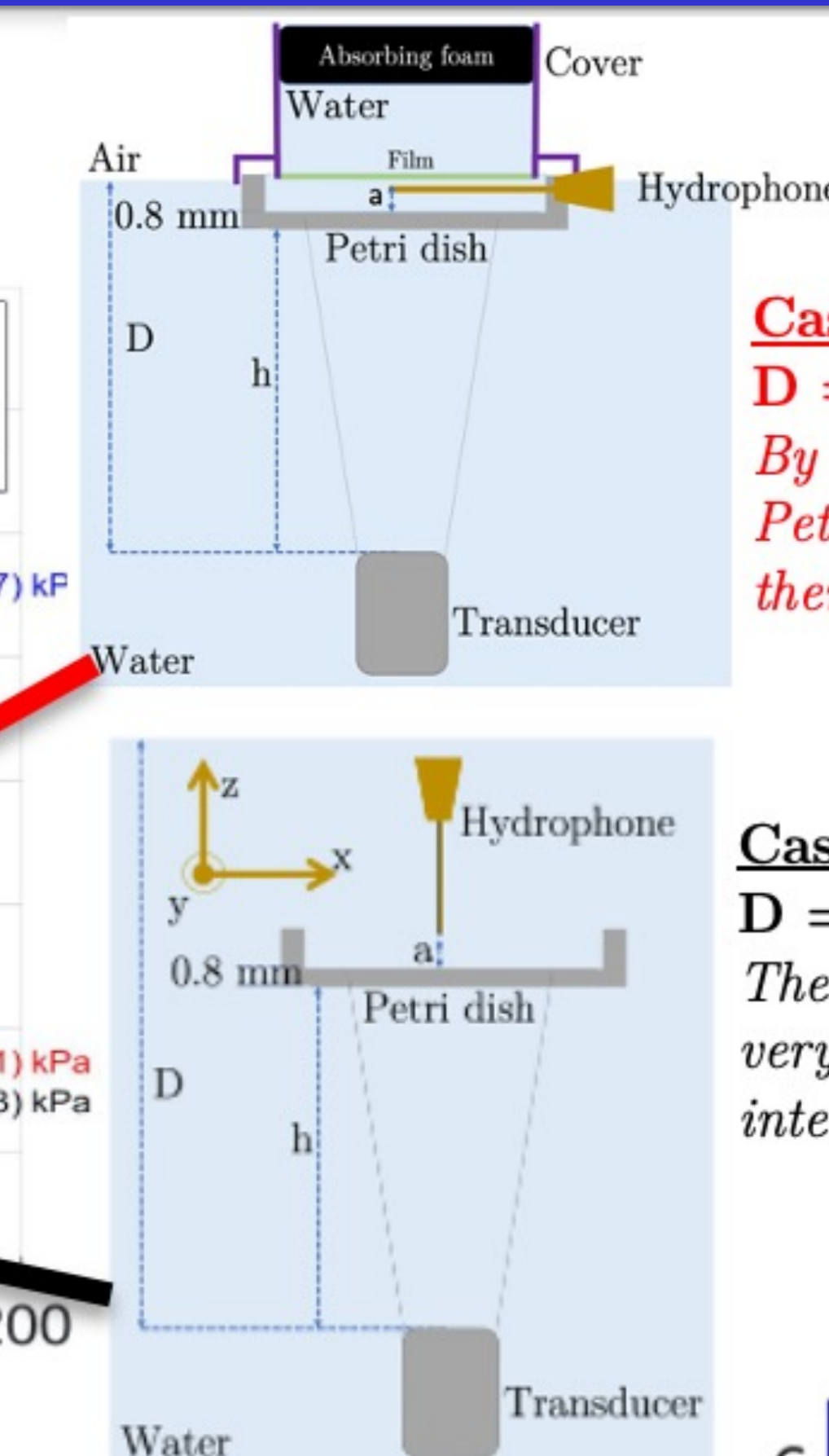
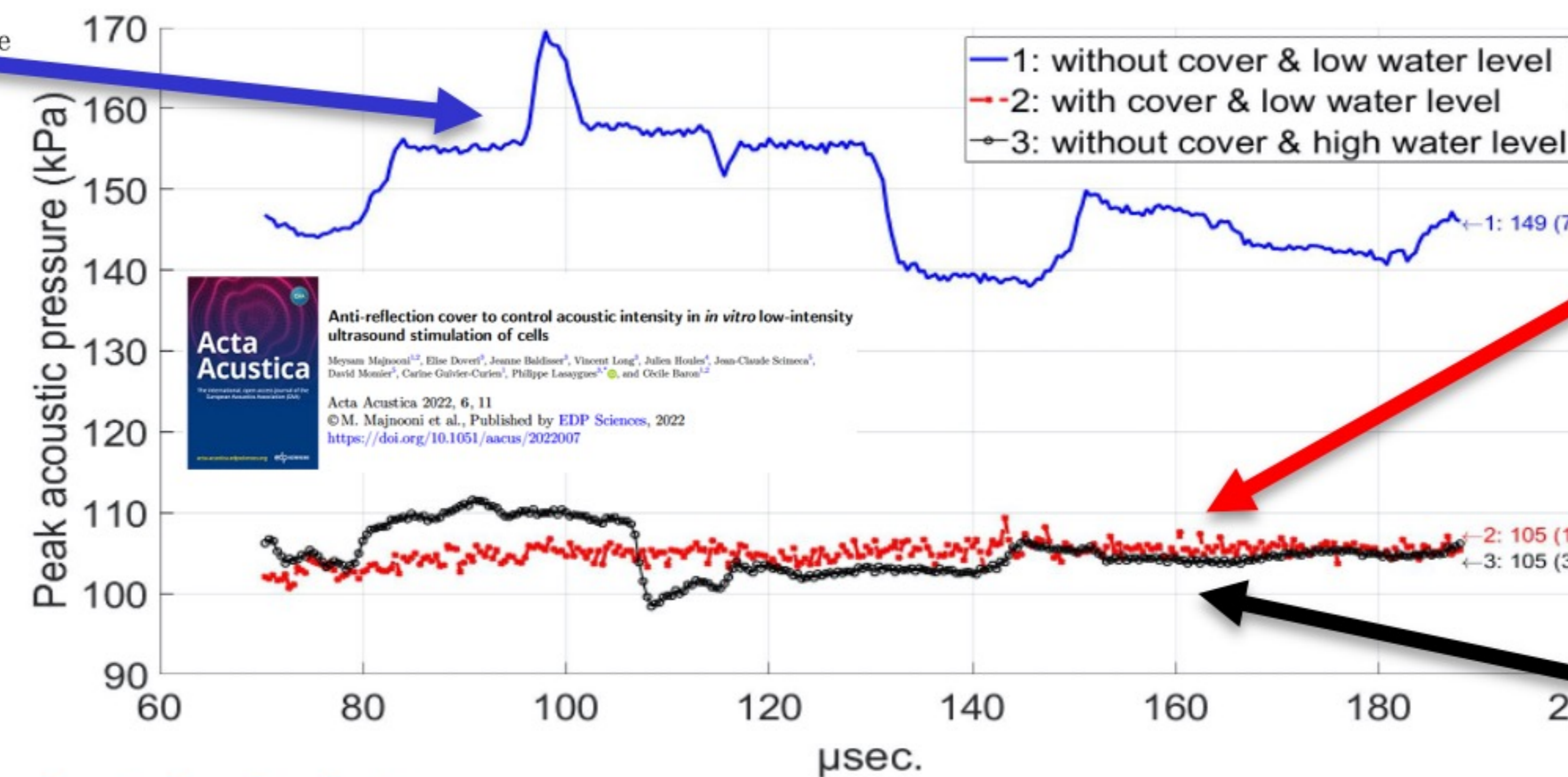
The **water PML (absorbing device)** is applied on the upper border of the culture medium. The absorbing device prevents formation of the standing waves inside the Petri dish (region A).



Acoustic intensity monitoring

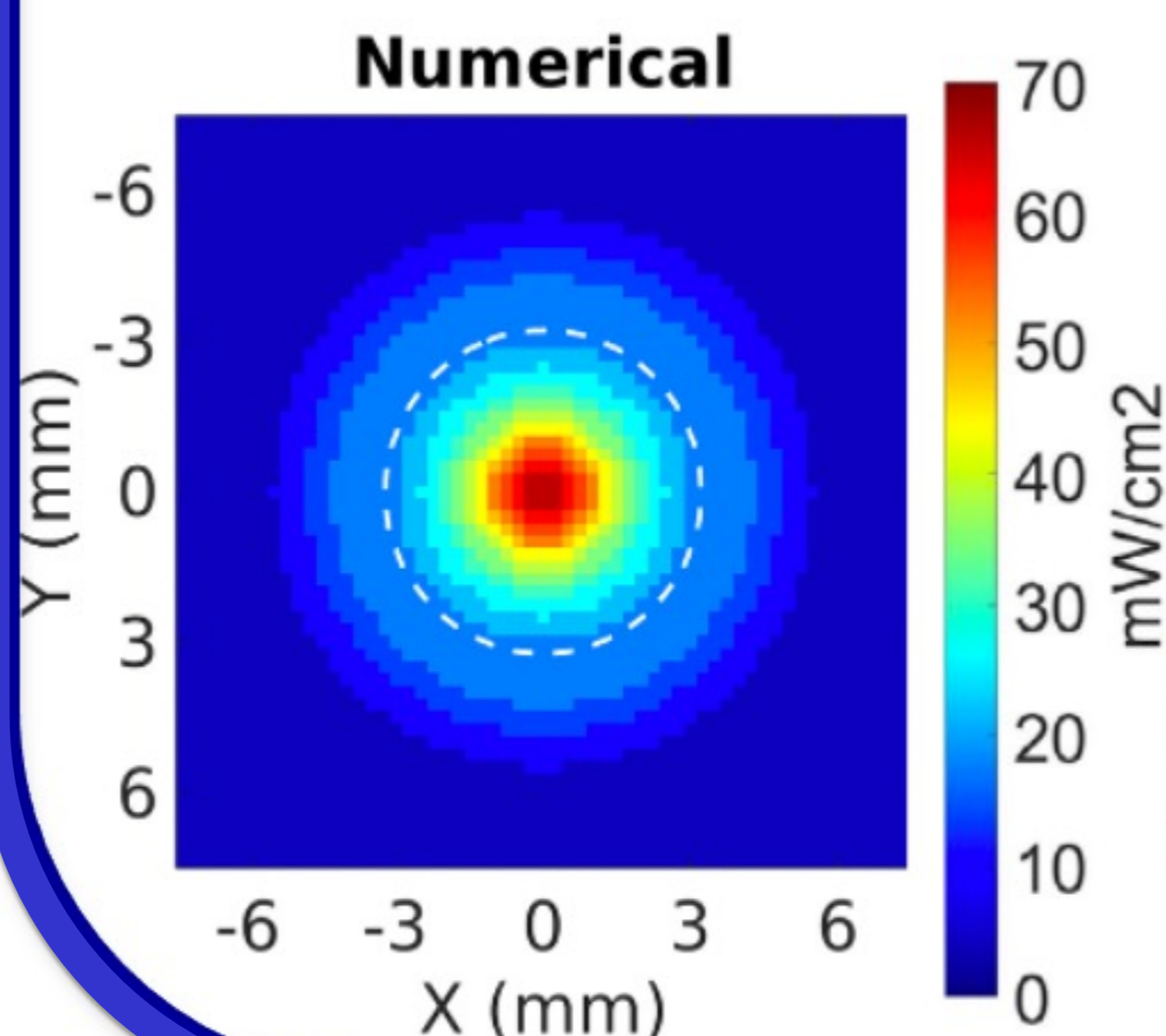


Case 1: without cover & low water level
 $D = 46.3$ mm
Existing standing waves in the water layer.



Case 2: with cover & low water level
 $D = 46.3$ mm
By placing the anti-reflection cover on the Petri dish, the waves are trapped and there are no more standing waves.

Case 3: high water level
 $D = 700$ mm
The water column above the Petri dish is very large, so the reflection at the water/air interface is far from the area of interest.



Analytical calculation

Intensity of the wave

Measured at 25 mm (under the Petri dish)		71.6
Measured at 28 mm (above the Petri dish)	$[I_{SPTA}]^M$	60.7
Calculated after 3 Runs in water		78.4
Transmitted into the Petri dish		73.5
Calculated after 3 Runs in the bottom thickness	$[I_{SPTA}]^A$	62.3

Numerical calculation

$$[I_{SPTA}]^N$$

(mW/cm²)

	71.6
	60.7
	78.4
	73.5
	62.3
	61.9

