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Methodological Approach to Define Low Intensity Pulsed Ultrasound Parameters Characterization for Cell Stimulation in Near and Intermediate Fields

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1. Introduction

Low-Intensity Pulsed Ultrasound (LIPUS) is a non-invasive technique increasingly used to stimulate cellular mechanotransduction in regenerative therapies (Majnooni et al., 2022). However, biological outcomes remain highly variable, partly due to incomplete characterization of acoustic exposure conditions. Most studies rely on Spatial-Average Temporal-Average Intensity (I_{SATA}), although the mechanical stimulus also depends on spatial and temporal parameters, including acoustic pressure (P_{peak}), Pulse Repetition Frequency (PRF), burst duration (t_{burst}), Duty Cycle (DC), and pause duration (t_{pause}), which enable cellular sensing and response and influence biological outcomes (Peñacoba et al., 2021; Orthodoxou et al., 2026). Standard descriptions often neglect spatial field distribution, despite differences between the near field, characterized by interference-induced fluctuations, and the intermediate field (Fung et al., 2014). These spatial variations affect cellular mechanical stimulation but are rarely considered. This study proposes a methodological approach to select acoustic parameters at constant I_{SATA} while accounting for propagation regime. Two temporal regimes are compared based on PRF and t_{pause} under identical I_{SATA}.

2. Methods

2.1 Experimental conditions

The acoustic field of a 1 MHz single-element transducer (V394-SU, Olympus Evident) was characterized in a degassed water tank using a needle hydrophone mounted on a 3-axis positioning system. Measurements at 30 mm (near field) and 150 mm (intermediate field) guided temporal parameter selection.

2.2 Acoustic parameter definition & framework

Instantaneous acoustic intensity $I = p^2(t)/(\rho c)$ was used to defin the Temporal-Average Intensity $I_{TA} = 1/T \int_0^T I dt$ for a pulsed wave. $I_{SATA} = \frac{1}{A}\int_A I_{TA}\, dA$ is I_{TA} averaged over the -6 dB beam area. For pulsed ultrasound, the Spatial-Average Pulse-Average intensity I_{SAPA} averages I_{TA} over a single burst and the beam area. The relation $I_{SATA} = I_{SAPA}\, DC$ where $DC = t_{burst}\, PRF$ enabled optimization at a constant I_{SATA} of 0.2 W/cm² in both fields. The pulse period is $T = 1/PRF$ and the pause duration is $t_{pause} = T - t_{burst}$. Cavitation risk and energy distribution were assessed by the Mechanical Index $MI = P_{neg} \cdot f^{-1/2}$ (P_{neg}=peak negative pressure) and the Beam Uniformity Ratio $BUR = I_{peak}/I_{mean}$, with f = frequency, $Ipeak$ = max(I_{TA}) and I_{mean} = spatial average of I. Interference was evaluated using the acoustic round-trip time $t_{TOF} = 2d/c$ (d = distance, c=sound speed). Peak pressure P_{peak} was also evaluated. To maintain constant I_{SATA}, the DC=20% for the intermediate field (Bohari et al., 2015) and 40% for the near field to compensate for lower pressures. Two temporal regimes were explored to compare contrasting pulse densities at identical I_{SATA}: (i) long pauses (t_{pause} = 1–10 ms; PRF 60–600 Hz near, 80–800 Hz intermediate), allowing partial recovery of the medium

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between pulses, and (ii) short pauses (t_{pause} = 0.2–0.8 ms; PRF 2–13 kHz near, 1–4 kHz intermediate), promoting cumulative mechanical exposure. Figure 1 summarizes the field parameters, analysis, and methodological framework for parameter selection. Acoustic fields were mapped by 2D lateral scans on a Cartesian grid (UMS Post-processing software) and post-processed in MATLAB® to extract selectionparameters.

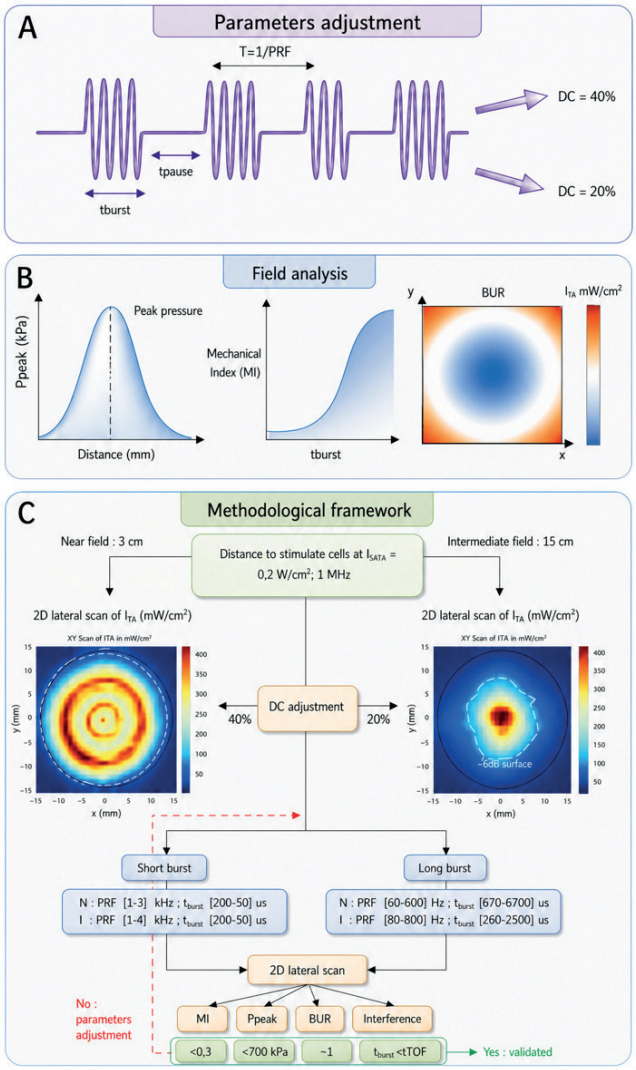

Figure 1. A. Parameters definition B. Field analysis and C. Methodological framework to define LIPUS stimulation condition, in near (N) and intermediate (I) field for cell stimulation.

Credit: Lucine PERLIN.

3. Results and discussion

The methodology enables comparison at constant I_{SATA}. Table 1 summarizes parameter selection in both fields, including cavitation safety (MI<0.3, P_{peak}<770 kPa), beam uniformity (BUR≈1), and temporal constraints (t_{burst}<t_{TOF} and t_{pause}). All configurations remained below cavitation thresholds. Higher MI near-field values reflect stronger local fluctuations from Fresnel-zone interference. BUR differed between regimes, with lower values in the near field indicating stronger spatial fluctuations and higher values in the intermediate field reflecting a more focused energy distribution. It should be considered a contrast indicator rather than a homogeneity metric. Temporal constraints were the main limitation. Short bursts satisfied t_{burst}<t_{TOF} in the intermediate field, whereas short-pause configurations were not achievable in the near field due to intrinsic interference. In the Fresnel zone, wave superposition generates pressure fluctuations that limit high-PRF compatibility. Phased arrays, apodization, and geometry optimization may mitigate these effects, although interference remains inherent to near-field propagation.

Table 1. Acoustic exposure parameters and field characterization in near (N) and intermediate (I) fields under constant I_{SATA}.

Field	t_{burst} (us)	PRF (kHz)	tpause (ms)	MI (-)	BUR (-)	P_{peak} (kPa)	t_{TOF} (us)
	Criteria			<0,3	≈ 1	<770	>t_{burst}
N	50	8	0,076	0,24	1,7	274	40
N	670	0,6	1	0,24	1,8	200	40
I	50	4	0,2	0,11	2	342	203
I	1500	0,133	6	0,11	2,1	303	203

Credit: Lucine PERLIN

4. Conclusion

We present a methodology for justified LIPUS parameter selection at constant I_{SATA} in near and intermediate fields. By integrating spatial and temporal criteria, it supports reproducible stimulation conditions. Stable conditions are harder to achieve in the near field due to intrinsic interference, whereas the intermediate field offers a more homogeneous propagation region. Future work will investigate whether cellular responses depend on spatiotemporal structuring parameters beyond I_{SATA}.

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Contributor Roles

LP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Resources, Validation, Visualization, Writing- original draft, Writing – review & editing; MO: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing; AB: Data curation, Formal analysis, Investigation, Resources; SB: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing; RR: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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