Thomas Hauser, Mathias S. Weyland*, David Jürgensen, Reyhaneh Hooshmandabbasi, Franco Guscetti, Caroline Maake, and Stephan Scheidegger

In-vitro modelling and measurement of focused ultrasound fields for use in sonodynamic therapy

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Abstract: Sonodynamic therapy, which combines low-intensity ultrasound with sonosensitizers, shows promising anti-cancer effects, yet its underlying mechanisms remain incompletely understood. To support systematic *in vitro* studies, we developed and characterized an ultrasound setup designed to expose cells to well-defined and reproducible conditions. The setup includes a water tank, a sample holder, and absorbing structures to minimize stationary waves. Hydrophone measurements were conducted to assess the ultrasound pressure field, and finite element method (FEM) simulations were used to complement these measurements. The simulations allow for insights into power and intensity distributions that are challenging to measure directly. Results showed good agreement between measured and simulated pressures, although discrepancies of up to 20% were observed in ring patterns present in the simulation, but absent in the measurements. The study highlights the importance of simulations for optimizing experimental design and interpreting measurement artifacts, particularly those created by the hydrophone itself.

Keywords: pressure acoustic modelling, acoustic pressure measurements, sonodynamc therapy

1 Introduction

Previous research provided evidence for remarkable anti-cancer effects of sonodynamic therapy, which combines low-intensity ultrasound with sonosensitizers [1, 2]. The underlying biophysical processes of this modality are still not fully resolved and may be related to thermal or sonochemical effects, as well as micro-mechanical strain and stress affecting cellular structures [3]. While more thorough studies are warranted, many published reports lack important details about experimental conditions – an issue that has also been criticized in the literature [4, 5]. As a prerequisite for future systematic *in vitro* experiments, an ultrasound setup was developed and characterized with the aim to expose cells to well-defined, reproducible conditions (temperature, wave pressure).

The characterization of the experimental setup is based on measurements of the pressure field using a hydrophone. To evaluate an optimal design of the sample holder, finite element method (FEM) simulations have been carried out. The benefits of modelling this setup are two-fold: First, a well-calibrated simulation framework allows for examinations of potential modifications or extensions of the physical setup before these modifications are implemented. Second, power and intensity within the tank are two critical values as they relate to the amount of energy deposited in the cells. Yet, measuring power and intensity at high spatial resolution is extremely challenging. An alternative route to insights is, however, to calibrate a simulation based on measured pressure data and then extract power and intensity from the simulation.

***Corresponding author: Mathias S. Weyland,** School of Engineering, Zurich University of Applied Sciences, Technikumstr. 9, 8401 Winterthur, Switzerland, mathias.weyland@zhaw.ch
Thomas Hauser, Stephan Scheidegger, School of Engineering, Zurich University of Applied Sciences, 8401 Winterthur, Switzerland
David Jürgensen, Reyhaneh Hooshmandabbasi, Caroline Maake, Institute of Anatomy, University of Zurich, CH-8057 Zurich, Switzerland
Franco Guscetti, Institute of Veterinary Pathology, University of Zurich, CH-8057 Zurich, Switzerland

2 Methods

The setup consists of a water tank, a sample holder, absorbing structures cast from Aptflex F36 (Precision Acoustics Ltd, Dorchester, United Kingdom) to avoid stationary waves, a frame for precise measurements in 3D, and an ultrasonic transducer built in-house operating at 1.1 MHz (Fig. 1) and driven with an electric power of 9820 mW. The water tank with 1 cm thick PMMA walls measures 20 cm in width and length and 15 cm in height and is filled with degassed Millipore water roughly to the 12 cm during characterization. A 3D-printed sample holder is inserted into the tank to position a flat bottom Corning Stripwell microplate (Corning Incorporated, Corning, NY, USA) which would contain cells subject to the ultrasonic treatment during *in vitro* treatments. A heater with built-in circulation pump (ProfiCook PC-SV 1126, Clatronic International GmbH, Kempen, Germany) is used to maintain a water

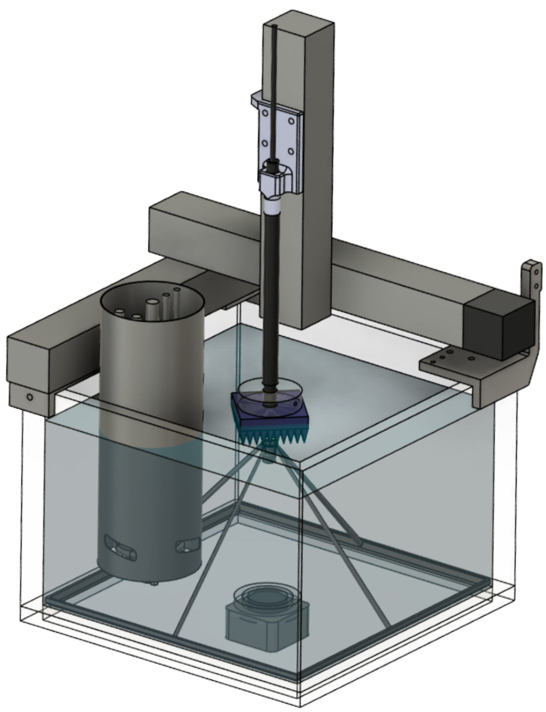

Fig. 1: Water tank setup with heater of cylindrical shape, sample holder of pyramidal shape, absorbing structures located above the holder, frame for hydrophone positioning and ultrasonic transducer and corresponding electronics at the bottom of the tank.

temperature of 37 °C in order to ensure a suitable environment during these treatments.

The pressure in the ultrasound field is measured with a hydrophone (Müller-Platte Nadelsonde, Müller Instruments, Oberursel, Germany) and compared to finite element method (FEM) simulations.The hydrophone used in this study features a delicate 1.2 mm diameter tip, yet of a size comparable to the wavelength in water ($\lambda \approx 1.35$ mm). To position the hydrophone within the tank, it is mounted on a 3D printed holder connected to a frame that registers with the tank (gantry). This gantry is equipped with stepper motors and homing switches to ensure reproducible measurements. All pressures reported in this work (i.e. measurements and simulation) are root-mean-square (RMS) pressures over one cycle of the acoustic wave.

The simulations are implemented in COMSOL Multiphysics® V. 6.3 (COMSOL AB, Stockholm, Sweden) and combine the piezoelectric and pressure acoustic modules of the software suite. The simulation is set up in a 2D-axisymmetric fashion; thus, the tank walls are assumed to be cylindrical. Although this is different in the real setup, prior simulation studies have revealed that the presence or absence of walls does not affect the field in any relevant manner due to the distance to the high-pressure region of the field. Material parameters from the COMSOL material library are used, the outer walls of the tank are implemented with hard boundaries, and the water-air interface is modeled with a soft boundary; perfectly matched layers are used for the absorbing structures. The voltage applied to the simulated transducer is scaled so that the average simulated pressure is equal to the measured pressure averaged across all slices. To assess the influence of the hydrophone, a simulation with omitted hydrophone is compared to one where the hydrophone is present.

3 Results

Fig. 2 shows a comparison between the measured pressure (top) and the simulated pressure (center) in a horizontal slice at a height of 90 mm from the tank floor, along with the pressure difference between measurement and simulation (bottom).

This comparison between measurement and simulation reveals that the pressures are generally in agreement with a mean difference of 1.02 $\pm$ 0.88 kPa (SD). Changes in the pressure field on the same slices between consecutive measurements are 3.7 kPa on average, and even lower in the hotspot area, with a mean difference of 0.49 kPa. Small shadows around the hotspot vary, but their influence on the center (hotspot) is not recognizable.

The results shown in Fig. 2 are from simulations without a hydrophone. The effect of the hydrophone is shown in Fig. 3, where the pressure on a slice along the vertical central axis of the tank is visible. The hydrophone is omitted in the top illustration and present in the center illustration. In the latter illustration, a standing wave pattern surrounding the hydrophone tip is clearly visible (red arrow). The difference between the two former images is shown at the bottom. In general, a focus zone is seen at a height of approx. 70 mm above ground, suitable for the placement of cells if a strong, but narrow field is desired.

4 Conclusion

The results reported in this work demonstrate general agreement between the measurements and the simulations. The measurements facilitate the choice of position of the cell sample concerning constraints such as absolute pressure values or homogeneity of the pressure field. The shape and width of the pressure field are as important as the pressure for a uniform treatment of the cells.

Furthermore, simulations can be used to investigate potential future modifications of the setup without the need of implementing the modifications and provide a means to quantify power and intensity within the tank – a physical quantity

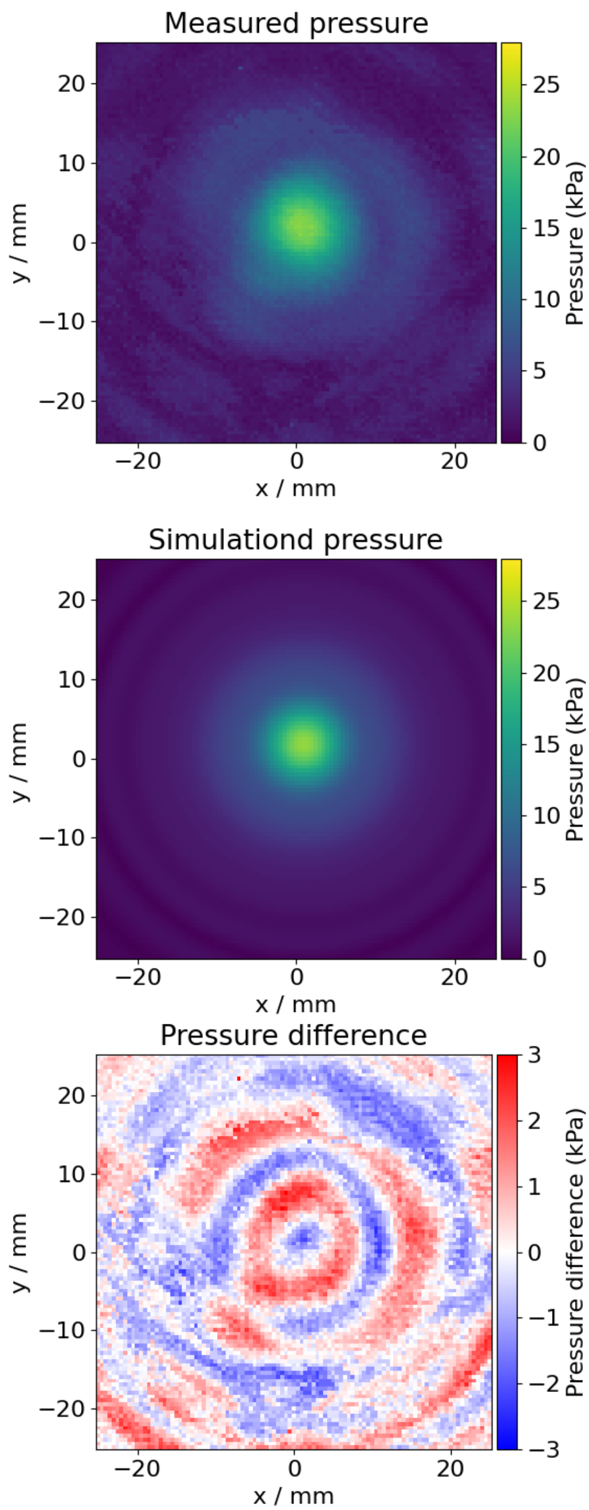

Fig. 2: Measured (top) and simulated (center) pressures on a horizontal slice at a height of 90 mm above the tank floor, along with the pressure difference between measurement and simulation.

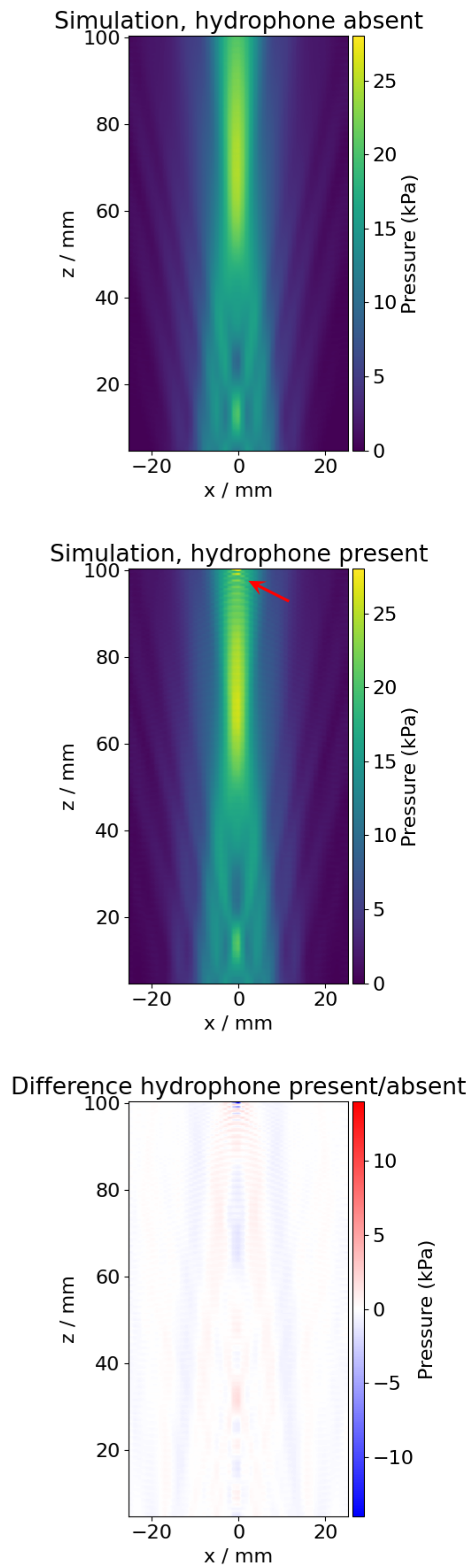

Fig. 3: Vertical slice extracted from simulation where hydrophone is omitted (top), in comparison to a simulation including the hydrophone (center) which generates a standing wave (red arrow). The difference image is shown at the bottom.

that cannot be measured easily directly. Yet, the simulation revealed that artifacts in the local wave field caused by the hydrophone call for caution when interpreting measured pressure figures. The simulations with and without the hydrophone can be used to relate the measurements using a hydrophone with the acoustic pressure field, which is experienced by the cells and not modified by the hydrophone. This is important since hydrophones, even when small, produce standing waves that lead to inhomogeneous conditions with respect to mechanical strain/stress, and temperature and therefore cannot be used during cell treatment.

Author Statement

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