INHOMOGENEITY OF ULTRASOUND FIELD DURING SONICATION EXPERIMENTS *IN VITRO* AND ITS INFLUENCE ON THE OVERALL AMOUNT OF ULTRASOUND ENERGY ENTERING A SONICATION VESSEL

Martin Snehota, Jaromir Vachutka, Ladislav Dolezal, Klara Balazova, Hana Kolarova

Department of Medical Biophysics, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic

Abstract

Current sonication experiments in vitro show immense variability in experimental set-ups and equipment used. Many factors such as presence of standing waves or position of sonicated sample in ultrasound field during the experiment affect ultrasound field parameters the sonicated samples actually experience. The main goal of this work was to quantify influence of position of sonicated sample on maximum acoustic intensity and overall amount of ultrasound energy entering sonication vessel when placed at different distances from ultrasound transducer. The measurements were performed in a water sonication tank with use of a circular unfocussed ultrasound transducer (d = 19 mm) and needle hydrophone (d = 0.5 mm). The measurements showed that the differences in amount of ultrasound energy (maximum and minimum energy were compared) entering particular well per time unit at different distances from ultrasound transducer range from 45.5% (48-well culture plate) to 109.9% (96-well culture plate). Moreover, the maximum acoustic intensity of ultrasound field entering particular well can differ by up to 233.2%. Therefore, position of sonicated sample in ultrasound field should not be neglected during sonication experiments in vitro.

Keywords

therapeutic ultrasound, in-vitro experiments, sonication vessel, ultrasound field structure

Introduction

Ultrasound (US) has been extensively used by clinicians as a powerful imaging method over the past decades. Potential uses of ultrasound in medical imaging have still not been fully exploited and many research teams and international companies focus on developing or improving new modalities such as ultrafast imaging, elastography, novel contrast agents or advanced techniques for imaging flow and tissue motion (e.g. B-flow, vector flow imaging or Doppler tissue imaging) [1]. Ultrasound has also been extensively studied for its therapeutic effects. Numerous powerful modalities such as HIFU (high-intensity focused ultrasound), ESWL (extracorporeal shockwave lithotripsy), phacoemulsification, tissue cutting and vessel sealing or various physiotherapy procedures have already found their way into clinical practice [2]. Other therapeutic methods are still being deeply studied. These include for instance drug delivery systems, gene therapy or sonodynamic therapy [3].

Prior to introducing a new therapeutic method, huge amount of testing and experiments is usually conducted *in vitro*, *in vivo* and in preclinical studies. Current sonication experiments *in vitro* show variable experimental set-ups that pose non-negligible uncertainty of ultrasound field parameters the sonicated samples actually experience. The most common experimental set-ups are depicted in Fig. 1 [4].

Commercial culture plates and dishes are often used as sample carriers, other research teams develop custom sonication vessels for ultrasound exposures. There have already been published two guidelines for proper reporting of ultrasound field parameters the sonicated samples experience during sonication [5, 6].

Lekar a technika – Clinician and Technology 2024, vol. 54(2), pp. 43–47, DOI: 10.14311/CTJ.2024.2.02
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

Both of them consider direct measurement of ultrasound field at the position of sonicated sample to be the most relevant when correlating biological response to ultrasound field parameters. Direct measurement can be sometimes impossible due to arrangement of the experimental set-up. In such case, the guidelines advise to measure ultrasound field at least in free field condition. The structure of ultrasound field generated by unfocussed transducers is usually defined by its near and far field [7]. Therefore, the actual amount of ultrasound energy entering particular sonication vessel may differ significantly depending on the position of sonicated sample in ultrasound field and dimensions of the sonication vessel.

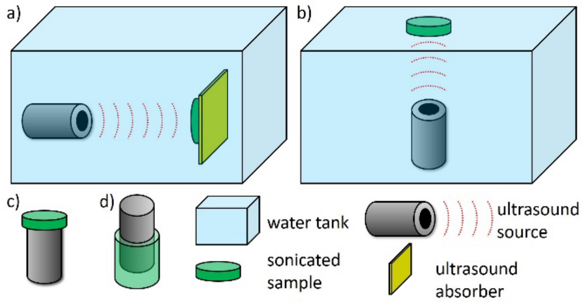

Fig. 1: Common experimental set-ups. a) ultrasound source and sonicated sample are both immersed in water sonication tank, ultrasound absorber is placed behind the sonicated sample, b) unsealed sonicated sample is placed at water level, ultrasound source is placed below it, c) sonicated sample is placed directly on the ultrasound source, ultrasound gel is usually used to couple ultrasound to region of interest, d) ultrasound source is immersed directly into the sonicated sample.

The main goal of this work was to determine differences in actual amount of ultrasound energy entering particular sonication vessel(s) when placed at different positions of ultrasound field. Dimensions of commercial 96-, 48-, 24-, 12- and 6-well culture plates (TPP, Switzerland) were considered during data analysis.

Methods

Equipment

All measurements were conducted in a water sonication tank filled with distilled water. Ultrasound field was generated with use of a circular plane piston unfocussed transducer SN: PA 192 (Precision Acoustics, United Kingdom) with nominal frequency of 3.5 MHz. The structure of ultrasound field was measured using a calibrated 0.5-mm needle hydrophone SN: 3474 (Precision Acoustics, United Kingdom). The hydrophone was positioned using a 3D positioning system that is integral part of the water sonication tank. The signal from hydrophone was registered with use of an oscilloscope WaveRunner 62Xi (LeCroy, New York, USA) and transferred to computer for further analysis. The manufacturer reports the overall uncertainty of the data measured by the hydrophone to be 17% at the levels of 3 and 4 MHz. All measurements were performed in free field condition.

Scans

Several types of scans were conducted during measurements. *X* axis corresponds to horizontal axis, *y* axis corresponds to vertical axis and *z* axis corresponds to distance from ultrasound transducer. Linear scan is constituted of measurements of acoustic parameters at particular points along one of the Cartesian axes. Orthogonal cross scan (OC) is constituted of two linear scans that mutually intersect in the middle. Planar scan is constituted of measurements of acoustic parameters at particular points that form a net along two of the Cartesian axes. Measurement scheme as well as types of scans are depicted in Fig. 2.

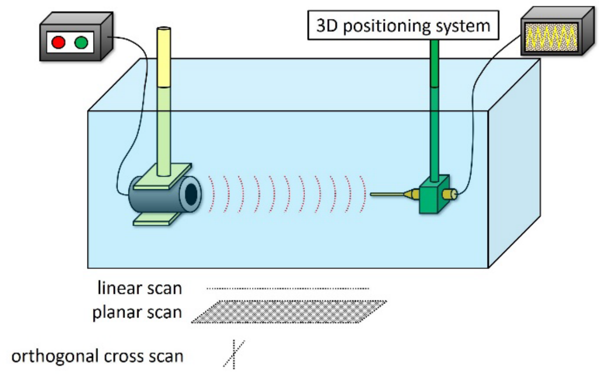

Fig. 2: Measurement scheme and types of scans.

Measurement procedure

The ultrasound transducer was precisely positioned to align its beam axis along the axis of hydrophone movement. The deviation of beam axis from axis of hydrophone movement was less than 0.5 mm at distance of $z = 440$ mm. Since the hydrophone had diameter of 0.5 mm, all scans were conducted with step of at least 0.5 mm to cover whole area of ultrasound field at particular distance. Linear scan along *z* axis was conducted to determine near field and far field region and locations of acoustic maxima and minima. Orthogonal cross scan and planar scan (both along *x* and *y* axis) were conducted at locations of acoustic maxima and minima. Even though the deviation of beam axis was small, the hydrophone was perfectly centralised on the beam axis at particular distances prior to conducting planar *xy* and OC *xy* scans.

Lekar a technika – Clinician and Technology 2024, vol. 54(2), pp. 43–47, DOI: 10.14311/CTJ.2024.2.02
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

Data analysis

Temporal average acoustic intensity I was determined for each of the measured points using UMS V1.5.2 software provided by the manufacturer. Firstly, the software determines the instantaneous pressure p through dividing the frequency components of the voltage signal registered via hydrophone V by hydrophone sensitivity M:

$$p(t) = \Im^{-1}\left\{\frac{\Im[V(t)]}{M(f)}\right\}$$

$\Im$ and $\Im^{-1}$ correspond to Fourier transformation and inverse Fourier transformation respectively. Pressure and voltage are time dependent, hydrophone sensitivity is frequency dependent.

Then, the value of root mean square pressure p_{rms} is calculated from the pressure waveform. Finally, the temporal average intensity I is calculated by dividing the square of p_{rms} by water density ρ and speed of ultrasound in water c:

$$I = \frac{{p_{rms}}^2}{\rho . c}$$

The surface area of particular well was superimposed over data of planar xy scans. Surface integration of intensities yielded acoustic energy per time unit P entering particular well at different distances. Maximum intensity entering particular well at different distances was also determined.

Results

Last axial maximum was found to be at distance of z = 199.25 mm. Linear z scan is depicted in Fig. 3. The orthogonal cross scans as well as planar scans (both along x and y axis) were conducted at distances of z = 0.00 mm, 14.75 mm, 17.75 mm, 21.75 mm, 27.25 mm, 33.75 mm, 44.25 mm, 64 mm, 99.25 mm, 199.25 mm (last axial maximum), 320.00 mm and 440.00 mm. Representative planar xy scans are shown in Fig. 4. Rest of scans can be found in Supplementary data. The ultrasound field generated by the transducer showed typical structure of ultrasound field described in literature [7]. Near field shows typical structure with strong local acoustic maxima and minima (z = 0.00–199.25 mm), whereas far field shows maximum acoustic intensity on the beam axis and its gradual decrease at periphery (z = 199.25–440.00 mm).

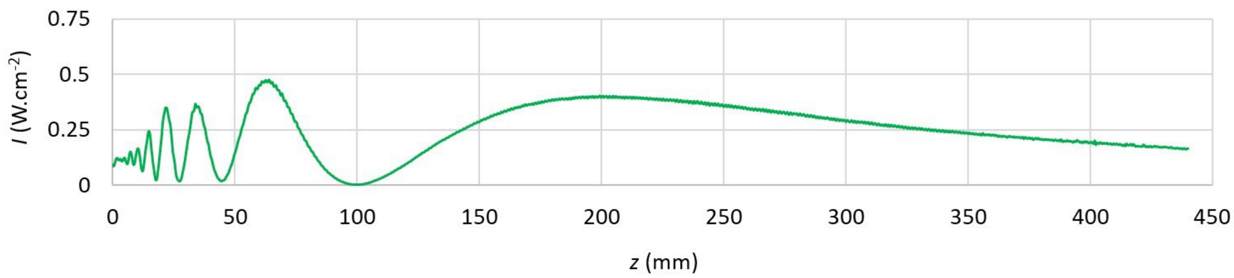

Fig. 3: Linear scan along z axis. The last axial maximum was found at distance z = 199.25 mm.

Table 1: Amount of ultrasound energy per time unit P and maximum acoustic intensity I_{max} entering particular well at different distances from ultrasound transducer. (wcp = well culture plate).*

	96-wcp* (d = 6.4 mm)		48-wcp* (d = 10.6 mm)		24-wcp* (d = 15.4 mm)		12-wcp* (d = 21.6 mm)		6-wcp* (d = 33.385 mm)	
z (mm)	I_{max} (W.cm^{-2})	P (W)	I_{max} (W.cm^{-2})	P (W)	I_{max} (W.cm^{-2})	P (W)	I_{max} (W.cm^{-2})	P (W)	I_{max} (W.cm^{-2})	P (W)
0	0.187	0.043	0.213	0.115	0.241	0.232	0.243	0.327	0.243	0.332
14.75	0.390	0.044	0.390	0.111	0.390	0.247	0.390	0.314	0.390	0.325
17.75	0.289	0.043	0.289	0.110	0.289	0.252	0.289	0.318	0.289	0.331
21.75	0.444	0.044	0.444	0.112	0.444	0.256	0.444	0.320	0.444	0.333
27.25	0.234	0.040	0.234	0.098	0.247	0.230	0.247	0.286	0.247	0.300
33.75	0.366	0.043	0.366	0.105	0.366	0.235	0.366	0.287	0.366	0.304
44.25	0.280	0.042	0.312	0.120	0.312	0.245	0.312	0.298	0.312	0.319
64	0.512	0.036	0.512	0.128	0.512	0.242	0.512	0.288	0.512	0.313
99.25	0.308	0.052	0.308	0.143	0.308	0.240	0.308	0.287	0.308	0.320
199.25	0.382	0.077	0.382	0.131	0.382	0.199	0.382	0.233	0.382	0.270
320	0.262	0.068	0.262	0.131	0.262	0.170	0.262	0.193	0.262	0.239
440	0.154	0.045	0.154	0.100	0.154	0.152	0.154	0.180	0.154	0.216
%	333.2	209.9	333.2	145.5	333.2	168.2	333.2	182.3	333.2	154.5

Lekar a technika – Clinician and Technology 2024, vol. 54(2), pp. 43–47, DOI: 10.14311/CTJ.2024.2.02
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

Table 1 summarizes the overall amount of ultrasound energy per time unit and maximum acoustic intensity entering particular well at different distances. The last line *%* corresponds to ratio between maximum and minimum value.

Data in Table 1 show several interesting results. When placed at different distances from ultrasound transducer, the maximum acoustic intensity of ultrasound field entering particular well can differ by up to 233.2% (z = 64 mm → 0.512 W.cm^{-2} compared to z = 440 mm → 0.154 W.cm^{-2}). These results are consistent for all wells. However, the difference of 233.2% results from spatial arrangement of our experiment. If the linear z scan was shorter in far field, the minimum intensity would be bigger and thus, the difference would decrease and vice versa, bigger distance from ultrasound transducer in far field would yield smaller acoustic intensity and the difference would increase.

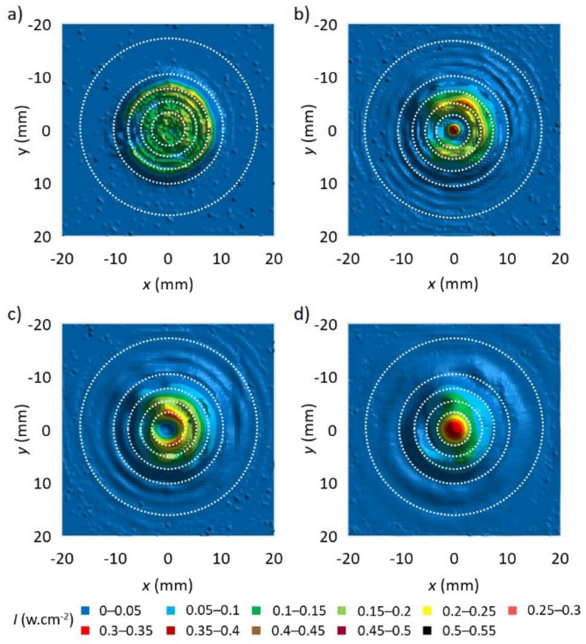

Fig. 4. Planar xy scans. The sizes of wells of 96-, 48-, 24-, 12- and 6-well culture plates are marked as white lines. a) z = 0.00 mm (right in front of the ultrasound transducer), b) z = 64.00 mm (at absolute axial maximum), c) z = 99.25 mm (at absolute axial minimum), d) z = 199.25 mm (at last axial maximum).

Moreover, the overall amount of ultrasound energy entering particular well per time unit can also differ significantly. When the maximum and minimum values of acoustic energy entering particular well per time unit at different distances are compared, the differences range from 45.5% (48-well culture plate) to 109.9% (96-well culture plate).

Discussion

Current sonication experiments *in vitro* show huge variations across experimental set-ups. During the experiments the sonicated samples are placed in different positions in ultrasound field. Due to the inhomogeneity of ultrasound field the amount of energy entering particular sonication vessel can differ significantly.

Based on the measurements we performed in our experimental conditions the amount of energy entering the well of 96-well culture plate per time unit can be more than 2 times bigger when placed at different distances from ultrasound transducer. Moreover, the maximum acoustic intensity differs more than 3 times for all culture plates. Therefore, the position of sonicated sample during the experiment should not be neglected as it may affect ultrasound field parameters the sonicated sample actually experiences and thus, it may affect final biological outcome.

The results presented in this study are specific for the equipment we used. There are many different commercially available therapeutic ultrasound systems with various ultrasound transducers. Moreover, various sonication vessels are used during the experiments. The variations of maximum acoustic intensities as well as amounts of ultrasound energy are specific for each of the systems and sonication vessels. Therefore, it may be beneficial to determine ultrasound field parameters within particular sonication vessel as recommended by ter Haar et al. [5] and Edmonds et al. [6].

There are also many other factors affecting ultrasound field parameters within particular sonication vessel than the position of the sonicated sample in ultrasound field. For instance, presence of standing waves results in formation of very strong acoustic maxima and minima which leads to huge inhomogeneity in local acoustic pressures [8, 9]. Therefore, the biological outcome of particular cells can be strongly variable depending on their position in the sonicated sample.

Transmissivity of coupling medium may also play a certain role. It has been shown that degassed water or gel-based media are more suitable for ultrasound coupling to region of interest than oils, cream-based preparations or tap water [10, 11]. Increasing distance between ultrasound transducer and sonicated vessel results in thicker layer of coupling medium increasing its potential influence on the dose of ultrasound energy delivered to region of interest.

Moreover, the interaction of ultrasound with sonication vessel can also change ultrasound field parameters in the sonication vessel significantly. For instance, glass culture dishes, plates and vessels reflect huge amount of ultrasound energy due to the acoustic impedance mismatch between distilled water and glass

Lekar a technika – Clinician and Technology 2024, vol. 54(2), pp. 43–47, DOI: 10.14311/CTJ.2024.2.02
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)

reducing the amount of ultrasound energy in the vessel significantly [12]. It has also been reported that the diffraction of ultrasound at round bottom of commercial culture plates and dishes results in formation of ultrasound field pattern with strong local acoustic maxima and minima corresponding to near field pattern [13].

Conclusion

The dimensions of sonication vessel and spatial arrangement of the sonication experiment affect the final amount of ultrasound energy entering particular sonication vessel. Direct measurement of ultrasound field parameters at the position of sonicated sample is considered to be the most relevant for correlating of biological outcome with ultrasound field parameters.

Acknowledgement

This work was supported by the grant project of The Ministry of Health of the Czech Republic - MZCR - NU21-09-00357.

References

[1] Hoskins P, Martin K, Thrush A, editors. Diagnostic ultrasound: physics and equipment. 3rd ed. Boca Raton, FL: CRC Press/Taylor & Francis Group; 2019. DOI: 10.1201/9781138893603

[2] Miller DL, Smith NB, Bailey MR, Czarnota GJ, Hynynen K, Makin IR, et al. Overview of Therapeutic Ultrasound Applications and Safety Considerations. Journal of Ultrasound in Medicine. 2012 Apr 1;31(4):623–34. DOI: 10.7863/jum.2012.31.4.623

[3] Escoffre JM, Bouakaz A, editors. Therapeutic Ultrasound. 1st ed. Cham: Springer International Publishing; 2016. 465 p. DOI: 10.1007/978-3-319-22536-4

[4] Alassaf A, Aleid A, Frenkel V. In vitro methods for evaluating therapeutic ultrasound exposures: present-day models and future innovations. Journal of Therapeutic Ultrasound. 2013 Dec;1:21. DOI: 10.1186/2050-5736-1-21

[5] ter Haar G, Shaw A, Pye S, Ward B, Bottomley F, Nolan R, et al. Guidance on Reporting Ultrasound Exposure Conditions for Bio-Effects Studies. Ultrasound in Medicine & Biology. 2011 Feb;37(2):177–83. DOI: 10.1016/j.ultrasmedbio.2010.10.021

[6] Edmonds PD, Abramowicz JS, Carson PL, Carstensen EL, Sandstrom KL. Guidelines for *Journal of Ultrasound in Medicine* Authors and Reviewers on Measurement and Reporting of Acoustic Output and Exposure. Journal of Ultrasound in Medicine. 2005 Sep 1;24(9):1171–9. DOI: 10.7863/jum.2005.24.9.1171

[7] Hobbie RK, Roth BJ. Intermediate Physics for Medicine and Biology. 4th ed. New York: Springer; 2007. 616 p. DOI: 10.1007/978-0-387-49885-0

[8] Hensel K, Mienkina MP, Schmitz G. Analysis of Ultrasound Fields in Cell Culture Wells for *In Vitro* Ultrasound Therapy Experiments. Ultrasound in Medicine & Biology. 2011 Dec;37(12):2105–15. DOI: 10.1016/j.ultrasmedbio.2011.09.007

[9] Leskinen JJ, Hynynen K. Study of Factors Affecting the Magnitude and Nature of Ultrasound Exposure with *In Vitro* Set-Ups. Ultrasound in Medicine & Biology. 2012 Mar 19;38(5):777–94. DOI: 10.1016/j.ultrasmedbio.2012.01.019

[10] Cage SA, Rupp KA, Castel JC, Saliba EN, Hertel J, Saliba SA. Relative Acoustic Transmission of Topical Preparations Used With Therapeutic Ultrasound. Archives of Physical Medicine and Rehabilitation. 2013 Apr 10;94(11):2126–30. DOI: 10.1016/j.apmr.2013.03.020

[11] Casarotto RA, Adamowski JC, Fallopa F, Bacanelli F. Coupling agents in therapeutic ultrasound: acoustic and thermal behavior. Archives of Physical Medicine and Rehabilitation. 2004 Jan;85(1):162–5. DOI: 10.1016/s0003-9993(03)00293-4

[12] Snehota M, Vachutka J, Dolezal L, Kolarova H, Mala Z, Zarska L. Analysis of ultrasound field parameters during sonication experiments in vitro—influence of laboratory glass and plastics. Lékař a technika – Clinician and Technology. 2017;47(4):113–21.

[13] Snehota M, Vachutka J, Dolezal L, Balazova K, Trneckova M, Kolarova H. Far field during sonication experiments in vitro – Is it really far enough? Ultrasonics. 2021;115:106461. DOI: 10.1016/j.ultras.2021.106461

prof. RNDr. Hana Kolarova, CSc.
Department of Medical Biophysics
Faculty of Medicine and Dentistry
Palacky University Olomouc
Hnevotinska 3, Olomouc, 775 15, Czech Republic

E-mail: hana.kolarova@upol.cz
Phone: +420 585 632 101

Lekar a technika – Clinician and Technology 2024, vol. 54(2), pp. 43–47, DOI: 10.14311/CTJ.2024.2.02
ISSN 0301-5491 (Print), ISSN 2336-5552 (Online)