Safety and dose estimation of transcranial focused ultrasound stimulation (TFUS)

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Safety and dose estimation of transcranial focused ultrasound stimulation (TFUS)

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by
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Co-supervisor: Associate Professor Lars G. Hanson
Co-supervisor: Assistant Professor Hyunjoo J. Lee

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## CONTENTS

<table>
<thead>
<tr>
<th>Preface</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>vii</td>
</tr>
<tr>
<td>Abstract</td>
<td>x</td>
</tr>
<tr>
<td>Resumé</td>
<td>xi</td>
</tr>
<tr>
<td>List of figures</td>
<td>xvii</td>
</tr>
<tr>
<td>List of tables</td>
<td>xix</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>xxi</td>
</tr>
</tbody>
</table>

### 1 Introduction

1.1 Aims of the project ............................................. 3
1.2 Thesis outline ................................................... 3

### 2 Background

2.1 Applications of ultrasound in medicine and science .............. 5
2.2 History of ultrasound neurostimulation ........................... 6
2.3 Basics of acoustics ................................................ 9
  2.3.1 What is a wave? .............................................. 9
  2.3.2 Acoustic variables ........................................... 10
  2.3.3 Medium acoustic property .................................... 12
  2.3.4 Tissue/wave interaction .................................... 13
  2.3.5 Wave equation .............................................. 17
2.4 Simulation environment ............................................ 19
  2.4.1 Simulation methods .......................................... 20
  2.4.2 Finite-differences time-domain methods ...................... 20

### 3 Safety of TFUS

25
4 Measurements for the validation of a modeling framework for realistic scenarios  
4.1 Setup for measurements in a pure water background  
4.2 Performed beam characterizations  
5 CMUT and magnetic resonance compatibility  
6 Conclusions and future perspectives  
6.1 Safety of TFUS  
6.2 Dose control  
6.3 CMUT-MRI compatibility  
6.4 Future work  
Bibliography  
Appendices  
Appendix A Safety of transcranial focused ultrasound stimulation: A systematic review of the state of knowledge from both human and animal studies  
Appendix B The Fallacy of Simplicity: Transducer modeling for accurate acoustic simulations of transcranial focused ultrasound stimulation  
Appendix C Physics based, validated reliable modeling of single element focused ultrasound transducer (SEFT)  
Appendix D Impact of the skull model on simulated TFUS beam profiles  
Appendix E Miniature ultrasound ring array transducers for transcranial ultrasound neuromodulation of freely-moving small animals  

This thesis is presented as a partial requirement for obtaining a PhD degree from the Technical University of Denmark (DTU). The project was funded by a DTU PhD stipend.

The research was carried out at the Department of Electrical Engineering (DTU Elektro) which came part of the new DTU Health Technology department from the 1\textsuperscript{st} of January 2019. The PhD project started on the 15\textsuperscript{th} of December 2015 and ended on the 14\textsuperscript{th} of June 2019. The study was carried out in collaboration with two different research centers: the Korea Advanced Institute of Science and Technology (KAIST), where I was hosted one year in the Brain/Bio Medical Microsystems (BMM) Lab, and the Danish Research Centre for Magnetic Resonance (DRCMR).

The PhD project was supervised by Associate Professor Axel Thielscher (main supervisor), Associate Professor Lars G. Hanson (co-supervisor) and Assistant Professor Hyunjoo J. Lee (co-supervisor).

Kongens Lyngby,  
14\textsuperscript{th} June 2019  
Cristina Pasquinelli
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I would like to thank Luca for his endless care and love.
And, above all, I thank my parents, Giorgio e Ornella, always with me.
Brain stimulation techniques can reversibly modulate the brain activity using different sources like pulsed magnetic field (transcranial magnetic stimulation, TMS) or electrical current (transcranial electrical stimulation, tES). With applications ranging from clinical to basic science, non-invasive approaches to induce neuronal effects are preferred, because of the lower risk-benefit ratio compared to invasive techniques. For specific intents, such as exploring the function of a defined area in the brain, and depending on the characteristics of the region of interest, the stimulation focus is required to be small, or deep, or both, which might be elusive for the majority of non-invasive brain stimulation techniques.

The employment of ultrasound waves as a source for neuromodulation, in a method called transcranial focused ultrasound stimulation (TFUS), has gained momentum in the last decade. TFUS holds the promise of a smaller spatial focus and the possibility to reach a deeper target in the brain compared to other non-invasive techniques. However, despite its proven excitatory and inhibitory effects on humans and animals, investigations to establish the method’s foundations are missing, although these studies are indispensable for safe and reliable usage of TFUS. In particular, the focuses of this study were to explore the therapeutic window which is the range within which the stimulation is safe and still effective, and the dose control, which is the actual dose that reaches the target after cranial transmission.

The first aim of the project was to collect information on the possible harmful effects of TFUS, with the goal of gaining knowledge on the upper limit of the therapeutic window, i.e. the threshold above which adverse effects start to appear. In particular, based on the available literature in animals and humans studies, the possible detrimental side effects and their causes were described together with their assessment in conjunction with the observed neural effect. TFUS appeared to be safe in most of the cases, except for a few animals in two studies, where signs of microhemorrhage were observed. Our review showed that further studies are necessary to demonstrate the reproducibility of the observed effect and to investigate its cause.
The second aim of the project was to find a procedure for dose control in complex and realistic scenarios with impossibility of ex-vivo measurements, for example after transcranial stimulation in humans experiment. We built an experimental setup to characterize the ultrasound propagation without and with obstacles in a pure water background, with the possibility to export these data into a simulation environment. While a common approach is to model the ultrasound transducer as a single piezoelectric element, we investigated a representation that considers the internal structure of the device, showing that the latter is necessary to have a better fit with the experimental data, in particular when obstacles are present. The obstacles were homogeneous 3D-printed objects and animal skull samples for which we developed a method for mapping their geometry and acoustic properties from computed tomography (CT) data, based on already existing approaches.

Finally, we were motivated to examine the possibility of combining TFUS with functional magnetic resonance (fMRI) to observe the network response to the stimulation and its dependency on the used parameters. In particular, we tested a capacitive micro-machined ultrasonic transducer (CMUT) designed for animals experiment in a 3T human scanner and we didn’t observe any measurable effects of the device on the MR images. However, radio frequency (RF) noise was measurable when the CMUT was powered.
Hjernestimulationsteknikker kan reversibelt modulere hjerneaktiviteten ved brug af forskellige kilder såsom magnetisk felt (transkraniel magnetisk stimulation, TMS), eller elektrisk strøm (transkraniel elektrisk stimulation, TES). Med deres anvendelse rangerende fra klinisk til grundlæggende videnskab, er ikke-invasive fremgangsmåder til at fremkalde neuronal effekt at foretrække, da de reducerer risiko relativt til fordele. For specifikke formål som f.eks. udforskning af funktionen af bestemte regioner i hjernen, og afhængig af de interessante regioners karakteristika, er målområder for stimulation nødvendigvis små, dybe eller begge, hvilket kan være svært at håndtere for størstedelen af de ikke-invasive hjernestimulationsteknikker.

Anvendelsen af ultralydsbølger som kilde til neuromodulation i en metode kaldet transkraniel fokuseret ultralydsstimulation (TFUS) har opnået fremgang det sidste årti. TFUS har potentiale til at stimulere et mindre fokus, og giver muligheden for at nå dybere målområder i hjernen sammenlignet med andre ikke-invasive teknikker. Til trods for metodens beviseligt stimulerende og hæmmende effekter i mennesker og dyr, savnes der undersøgelser for at etablere metodens grundlag, selvom sådanne er uundværlige for sikker og pålidelig anvendelse af TFUS. I særdeleshed har dette studies fokus været at udforske det terapeutiske vindue, som er grænserne for sikker og effektiv stimulation, samt dosis kontrol, som er den aktuelle dosis opnået efter kranial transmission.

Det første formål med projektet var at indsamle information om mulige skadelige effekter af TFUS med det formål at opnå viden om den øvre grænse af det terapeutiske vindue, dvs. tærskelværdier over hvilke ugunstige effekter opstår. Baseret på tilgængeligt litteratur vedrørende dyre- og humanstudier blev mulige skadelige bivirkninger og deres årsager vurderet sammen med de observerede neurale effekter. TFUS forekommer sikker i de fleste tilfælde, dog ikke for enkelte dyr i to af studierne, hvor blødninger blev observeret. Vores evaluering viste, at yderligere studier er nødvendige for at demonstrere reproducerbarheden af de observerede effekter samt undersøge årsagerne.
Det andet formål med dette projekt var at etablere en procedure for dosiskontrol i komplekse og realistiske scenarier, hvor ex-vivo målinger en umulige f.eks. efter transkraniel stimulation i humane eksperimenter. Vi har bygget en eksperimenter opstilling for at karakterisere ultralyds udbredelse med og uden forhindringer i en ren vandbaggrund med muligheden for at eksportere disse data til et simuleringsmiljø. Mens en almindelig indgangsvinkel er at modellere en ultralydstransducer som et enkel piezoelektrisk element, har vi undersøgt en beskrivelse, som inddrager de indre strukturer af udstyret, samt viser, at sidstnævnte er nødvendige for at opnå en bedre overensstemmelse med eksperimentelle data - i særdeleshed når forhindringer forekommer. Forhindringerne var homogene 3D-printede objekter og udsnit af dyrekrani for hvilke vi udviklede metoder til at kortlægge deres geometri og akustiske egenskaber fra CT skanningsdata (røntgentomografi) baseret på eksisterende teknikker. Afslutningsvis var vi motiverede til at undersøge muligheden af TFUS kombineret med funktionel magnetisk resonans (fMRI) for at observere netværksrespons til stimulationen og dens afhængighed af de anvendte parametre. Vi testede specifikt en kapacitiv mikro-fabrikeret ultrasonisk transducer (CMUT) designet til dyreeksperimenter i en 3T humant scanner og observerede ingen effekter af udstyret på MR billederne. Imidlertid var RF støj målelig, når transduceren var tændt.
PUBLICATIONS

Paper I

Safety of transcranial focused ultrasound stimulation: A systematic review of the state of knowledge from both human and animal studies

Cristina Pasquinelli, Lars G. Hanson, Hartwig R. Siebner, Hyunjoo J. Lee, Axel Thielscher

Submitted to Brain Stimulation and currently under revision.

Paper II

The Fallacy of Simplicity: Transducer modeling for accurate acoustic simulations of transcranial focused ultrasound stimulation

Cristina Pasquinelli, Hazael Montanaro, Hyunjoo J. Lee, Lars G. Hanson, Niels Kuster, Hartwig R. Siebner, Esra Neufeld, Axel Thielscher

Submitted to Journal of Neural Engineering

Paper III

Miniature ultrasound ring array transducers for transcranial ultrasound neuromodulation of freely-moving animals

Hyunggug Kim, Seongyeon Kim, Nam Seok Shim, Cristina Pasquinelli, Axel Thielscher, Jeong Ho Lee, and Hyunjoo J. Lee

Published in Brain Stimulation, volume 12, issue number 2, pages 251-255, year: 2019, doi: https://doi.org/10.1016/j.brs.2018.11.007.

Contributions to papers

This section will summarize my contribution to each paper.
Paper I

- Screening, reading of papers and collection of all data and information.
- Writing the manuscript, including preparation of figures and tables.

Paper II

- Performing all the measurements to characterize the beam in a pure water background with and without the obstacles. Analysis of the acquired data to prepare the intensity maps for the comparison with the simulations results.
- At KAIST, modification of the setup, data acquisition, and processing for pure water background measurements to perform data in the presence of obstacles. Supervision of Simon Danielsen, a student recruited to help in designing the holders for the transducer and the obstacles.
- Replication of the whole setup at DRCMR, with the help of Simon Danielsen for the metal and wood frames which surround the water tank and hold the stepper-motor system.
- Contribution to the acquisition of the CT data and processing the images to import in the simulation environment.
- Running the initial simulations, which revealed the need for a more systematic workflow to model the transducer and to map the acoustic properties from CT data. Our collaborators at ITIS foundation worked on the final simulation framework.
- Writing the paper and preparing the figures.

Paper III

- Helping in the acquisition and the processing of the MRI data and in writing the relevant part.
OTHER PUBLICATIONS

Impact of the skull model on simulated TFUS beam profiles
Cristina Pasquinelli, Hazael Montanaro, Esra Neufeld, Hyunjoo J. Lee, Axel Thielscher
Poster at 40th International Engineering in Medicine and Biology Conference (EMBC)
Honolulu, Hawaii, July 17-21, 2018

Physics based, validated reliable modeling of single element focused ultrasound transducer (SEFT)
Cristina Pasquinelli, Hazael Montanaro, Hyunjoo J. Lee, Niels Kuster, Esra Neufeld, Axel Thielscher
Poster at 19th International Symposium of ISTU - 5th European Symposium of EUFUS
Barcelona, Spain, June 13-15, 2019

Pitfalls in Acoustic Transducer Modeling for Focused Ultrasound (FUS)
Cristina Pasquinelli, Hazael Montanaro, Esra Neufeld, Redi Poni*, Niels Kuster, Axel Thielscher
Presented at 32nd Annual Meeting of the European Society for Hyperthermic Oncology (ESHO)
Berlin, Germany, May 16-19, 2018

Investigation of fallacies in focused ultrasound transducer acoustic modeling
Hazael Montanaro, Esra Neufeld, Niels Kuster, Cristina Pasquinelli, Lars G. Hanson, Axel Thielscher, Hyunjoo Jenny Lee
Presented at: The 18th International Symposium for Therapeutic Ultrasound (ISTU)
Nashville, Tennessee, May 14-17, 2018
LIST OF FIGURES

2.1 A qualitative representation of the different number of papers published in diagnostic ultrasound and in low intensity ultrasound modulation. .................................................. 8
2.2 The two types of acoustic waves, longitudinal and transverse. .......... 9
2.3 Illustration of particle displacement. ...................................................... 11
2.4 Reflected and transmitted waves. .......................................................... 14

4.1 Schematic of the setup for measurements in the water tank. .......... 29
4.2 The linear dependency of the measured voltage on the peak-to-peak voltage applied to the transducer. ........................... 30
4.3 The water tank and the screw stepper motors employed to characterized the ultrasound beam in a water background. .......... 31
4.4 The signal from oscilloscope before and after the application of a high pass filter. ................................................................. 32
4.5 Example maps of intensity, normalized to the maximum. ................. 33
4.6 An example intensity map and the relative phase map. ................. 33
4.7 Some of the obstacles placed between the transducer and the hydrophone to study how they interfere with the ultrasound beam. ... 34
4.8 Example intensity maps without and with the presence of skulls. .... 34

5.1 The packaged CMUT. ................................................................. 36

6.1 Different examples of how CT parameters and the type of background (water vs. air) can change the SNR of the images. ................. 40
6.2 Examples of EPI images with the CMUT in place. ......................... 41
6.3 Examples of B0 field images with the CMUT in place. ..................... 42
6.4 Setup to acquire the beam profile of the CMUT in an oil background. 42
6.5 An example of the intensity maps of CMUT under rapeseed oil. .... 43
LIST OF TABLES

1.1 Comparison between three brain stimulation modalities, including TFUS. .................................................. 2

4.1 The parameters used for the water tank measurements. ............... 32
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBB</td>
<td>blood-brain barrier.</td>
</tr>
<tr>
<td>CMUT</td>
<td>capacitive micro-machined ultrasonic transducer.</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography.</td>
</tr>
<tr>
<td>FDA</td>
<td>food and drug administration.</td>
</tr>
<tr>
<td>FDTD</td>
<td>finite-difference time-domain.</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging.</td>
</tr>
<tr>
<td>HIFU</td>
<td>high intensity focused ultrasound.</td>
</tr>
<tr>
<td>I_{sppta}</td>
<td>spatial peak temporal average intensity.</td>
</tr>
<tr>
<td>I_{spppa}</td>
<td>spatial peak pulse average intensity.</td>
</tr>
<tr>
<td>MI</td>
<td>mechanical index.</td>
</tr>
<tr>
<td>NIBS</td>
<td>non-invasive brain stimulation.</td>
</tr>
<tr>
<td>PDE</td>
<td>partial differential equation.</td>
</tr>
<tr>
<td>SEFT</td>
<td>single element focused transducer.</td>
</tr>
<tr>
<td>TFUS</td>
<td>transcranial focused ultrasound stimulation.</td>
</tr>
<tr>
<td>TI</td>
<td>thermal index.</td>
</tr>
<tr>
<td>US</td>
<td>ultrasound.</td>
</tr>
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</table>
CHAPTER ONE

INTRODUCTION

Low intensity transcranial focused ultrasound stimulation (TFUS) is a technique to stimulate the brain in both excitatory [1] and inhibitory [2] ways, with a transient or lasting, but still reversible, effect [3]. Brain stimulation techniques can be divided into two main groups: invasive and non-invasive. Invasive techniques have the advantage of a better spatial localization with the possibility to reach deep areas in the brain. However, they require surgery and therefore they are not the preferable choice for non-clinical humans experiment. On the other hand, non-invasive brain stimulation (NIBS) techniques have worse spatial precision and less depth of penetration. TFUS is a NIBS modality, which offers smaller focus size and a higher depth of penetration (Table 1.1) compared to other NIBS techniques. Table 1.1 shows a comparison between TFUS, transcranial magnetic stimulation (TMS), and deep brain stimulation (DBS), in terms of energy delivered, invasiveness, stimulation source and configuration, spatial resolution and depth of penetration. Moreover, contrary to other NIBS modalities, TFUS can directly evoke action potentials in a defined 3D target, that usually has an ellipsoid shape. In addition, TFUS is also attractive because it can be readily combined with other modalities such as functional magnetic resonance imaging (fMRI) or electroencephalography (EEG) without interfering with the recordings, as it applies acoustic waves rather than electric or magnetic fields. Along with basic science, intriguing clinical applications might be epilepsy treatment [4, 5] or an improvement in stroke recovery [6].

Given its promising features and possible applications, interest in TFUS has increased in the last decade. However, further investigations on specific problems are indispensable to move TFUS from initial pilot studies towards broader testing in humans in-vivo. In particular, a comprehensive safety profile is necessary to define
### TABLE 1.1: A comparison between different brain stimulation modalities is shown. The modalities are deep brain stimulation (DBS), transcranial magnetic stimulation (TMS) and transcranial focused ultrasound stimulation (TFUS). Adapted from [7].

<table>
<thead>
<tr>
<th></th>
<th><strong>DBS</strong></th>
<th><strong>TMS</strong></th>
<th><strong>TFUS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy delivered</strong></td>
<td>Electrical</td>
<td>Magnetic</td>
<td>Mechanical (most likely)</td>
</tr>
<tr>
<td><strong>Invasiveness</strong></td>
<td>Invasive</td>
<td>Non-invasive</td>
<td>Non-invasive</td>
</tr>
<tr>
<td><strong>Stimulation source</strong></td>
<td>Voltage/current source + electrical conducting probe</td>
<td>Alternating magnetic field</td>
<td>Low intensity pulsating ultrasound</td>
</tr>
<tr>
<td><strong>Stimulation configuration</strong></td>
<td>Implantable electrodes</td>
<td>Magnetic coils</td>
<td>Ultrasound transducer</td>
</tr>
<tr>
<td><strong>Spatial resolution</strong></td>
<td>Fractions of mm</td>
<td>~ 1 cm</td>
<td>2-5 mm</td>
</tr>
<tr>
<td><strong>Depth of penetration</strong></td>
<td>Unlimited</td>
<td>~ 1-1.5 cm unless H coil is used</td>
<td>10-15 cm or more</td>
</tr>
</tbody>
</table>

The *therapeutic window*, which is the dose range where the treatment is still effective without being harmful. Although for clearance of diagnostic and therapeutic ultrasound systems, a Food and Drugs Administration (FDA) guide exists [8] that is mandatory for commercial products in the USA, applying it to TFUS might be a conservative choice. In fact, a study on the dose-response of TFUS [9] showed that applying intensities above the values allowed in this guidance might be necessary to have a robust neural effect. The FDA guideline [8] is not based on risk-versus-benefit analysis [10], meaning that exceeding these limits will not necessarily result in an unsafe application of ultrasound. An accurate and precise control of the ultrasound dose, which is the actual dose that reaches the target in the brain, is necessary to determine the TFUS safety upper limits, and to ensure that they are not exceeded.

While the characterization of ultrasound beams in water background for diagnostic purposes is properly regulated (for example by the International Electrotechnical Commission standard IEC 62127-1), measurement of ultrasound propagation through a heterogeneous structure like the skull, as in TFUS, is not standardized. Adopting only these standards before TFUS application is not enough to control the dose because pressure attenuation and possible differences in the shape of the beam before and after cranial transmission are not taken into account. The skull is not homogeneous, but it is composed of three layers, two outer layers of cortical bone with a layer of cancellous bone inside, each having different density and thickness. Since human skulls have intra- and inter-individual variability in geometry and composition, personalized estimations are necessary. Computer simulations have been used to retrospectively simulate the intensity in the target, giving the skull the property of a homogeneous layer of cortical bone and acquiring CT data to derive its geometry [1, 11]. The ultrasound source, the transducer, is often modeled...
as a single piezoelectric element and adapted to fit the measurement [12]. However, this might not be the preferable approach when the skull is present.

To find the therapeutic window, animal studies are necessary because they allow ex-vivo assessment of the anatomical changes following TFUS. For small animal models, like mice or rats, a small, light and portable device is desirable because the animals can be stimulated while free to move and without anesthesia, which can interfere with the outcome of the experiment [13]. Functional magnetic resonance imaging (fMRI) is a valuable tool to quantitatively measure the network activation after TFUS, at a better spatial resolution compared to other non-invasive recordings techniques. Capacitive micromachined ultrasonic transducers (CMUT) are a valid alternative to piezoelectric transducers because they meet the criteria just mentioned of being small, light and portable. However, the combination of CMUT and fMRI has not been investigated yet.

1.1 Aims of the project

The aims of this project were:

✓ collecting the available data on TFUS safety to allow further investigation of the therapeutic window. A review has been conducted concerning the possible harmful effects and how to assess them, together with a description of the used parameters in relation with observed neural effects and the known safety limits for diagnostic and therapeutic ultrasound.

✓ a study to improve the prediction of the ultrasound post cranial beam transmission to allow a better dose control was performed. A setup which allows repeatable measurement in a water background and with obstacles in different positions has been used to measure the beam before and after transmission through different obstacles. These data allowed the validation of the transducer model and insight into acoustic mapping from CT data.

✓ understanding the steps necessary to make a CMUT device MRI-compatible.

1.2 Thesis outline

Chapter 1 gives an introduction to TFUS and why interest in it is increasing. The chapter presents also a description of the motivations underlying this thesis and the aims of the project.
CHAPTER 1. INTRODUCTION

In chapter 2 a brief overview on the historical steps which brought to today’s growing application of TFUS is presented. This chapter also gives a brief presentation of other ultrasound applications, to give an overview of its wide application. The basics of acoustics, including wave equations, acoustic variables, acoustic medium properties, and tissue/wave interaction are presented. Also, the software environment used for this project is presented.

Chapter 3 presents a work on the description of the potential harmful effects of TFUS and on how to assess possible unwanted damages following TFUS in both animal and human experiments.

Chapter 4 presents a detailed description of the setup for the characterization of ultrasound beams in a water background and the measurements used to improve the transducer model.

Chapter 5 presents our work on CMUT MRI compatibility.

Chapter 6 presents a summary of the main findings of the three different parts of this thesis (safety of TFUS, dose control, and CMUT-MRI compatibility), together with future perspectives.
2.1 Applications of ultrasound in medicine and science

Ultrasound waves can have two main effects on tissues: thermal (tissue heating by dissipation of energy in the tissue) or non-thermal (mainly of mechanical type), depending on the applied intensities. Appendix A gives a more detailed description of them.

High intensity focused ultrasound (HIFU) has a prominent thermal effect, and it can cause irreversible damage to tissue by rapidly raising the temperature to a level at which thermal necrosis occurs (above 56°C) [14]. Since the beam is focused, the region of high intensity is very small, and the surrounded area is relatively unaffected. For this reason, HIFU can be employed for tumors or ablation of abnormalities, both in a non-invasive way (for example for targeting the liver or the brain) and an invasive way (for example for prostate cancer treatment) [14]. High temperature can also be applied to specific areas in the brain to help patients with movement disorders, for example for tremor treatment after thalamotomy induced by HIFU [15].

Low intensity focused ultrasound (LIFU) has, on the contrary, a prominent mechanical effect. For example, it can be combined with a micro-bubbles contrast agent to temporarily and locally open the blood-brain barrier (BBB) by safely widening its tight junctions [16]. The BBB protects the brain from toxins, acting as a filter for the substances in the blood vessels. While its correct functioning is essential to maintain the homeostasis of the neuronal microenvironment, specific situations
require a temporary opening of this barrier to allow substances that otherwise would not pass through it, to reach the brain. For example, drug delivery in the brain is limited because only small-molecule drugs can cross the BBB in a pharmacologically significant amount, excluding 98% of small molecule drugs and approximately 100% of large-molecule neurotherapeutics to enter the brain [16]. This reduces the effective treatments to only a few diseases such as depression, chronic pain, and epilepsy [16]. Temporary opening of the BBB can therefore help therapies for a wide range of diseases and disorders -like Alzheimer’s, stroke, or tumors- by delivering therapeutics that usually do not reach the brain. For example, in brain tumor treatment, BBB opening might be necessary because cancer cells and small metastatic seeds might be protected by the BBB of the surrounding normal tissue, contrary to a primary and metastatic tumor that lacks a full-formed BBB [16].

While the previous therapeutic applications of ultrasound take advantage of the thermal or mechanical biophysical effects of ultrasound on tissue, the opposite, i.e. how tissues affect the path of the ultrasound wave, can be viewed as the basis for diagnostic ultrasound imaging [17]. The basic idea is to send pulsed ultrasound waves into the body, and reconstruct its internal structure through the received echos, which depend on the shape and composition of the encountered anatomical structures. Since ultrasonic diagnostic devices offer a portable and low priced tool for human body imaging, they have a wide range of clinical applications, for example tumor and internal organ abnormality diagnosis, or intraoperatively, where the transducer is placed in the body during surgery to localize the lesion.

Even though TFUS belongs to the therapeutic applications of ultrasound, given the wide use of diagnostic ultrasound and the rather extensive reviews and guidelines on its safety, the basis of TFUS has been built on this knowledge. In particular, the Food and Drugs Administration (FDA) guideline on the clearance of the selling of commercial diagnostic ultrasound devices state the upper limits of safety indices. These indices identify the risk of the potentially harmful effects on tissues after ultrasound exposure. These indices are mechanical index (MI), thermal index (TI), spatial peak pulse average intensity (I_sppa), and spatial peak temporal average intensity (I_stpa). A more detailed description of these indices can be found in the next chapters.

### 2.2 History of ultrasound neurostimulation

The spreading of ultrasound technology started with the demonstration of the piezoelectric effect, by the Curie brothers in 1880. After this, several technologies exploited this effect for several aims, for example for SONAR or metal flaw detection. The first detrimental effect of ultrasound wave on biological tissue was observed by
Langevin in 1920s: he showed that fish died when the tank where they were im-
mersed in was sonicated by high-intensity ultrasound [18]. In the same decade, the
first neural effect of ultrasound was documented when Harvey observed twitching
of gastrocnemius muscle after ultrasonic irradiation of sciatic nerve in frogs [19].
After 30 years, in the 1950s, the Fry brothers demonstrated a reversible modulation
of visual evoked potentials (VEPs) in cats [19]. From that year to the 2000s only
a few papers were published on the effect of ultrasound in the nervous system. In
particular, from the 1970s to the 1990s the main research areas were the effect of
ultrasound in stimulating the peripheral mechanical receptor underneath the skin
and in the auditory system in both humans and animals, the examination of the
US effects on peripheral and central nerve fibers, and the effects of US on brain
excitability in rats and rabbit [19]. This work was mainly performed by Gavrilov,
Vykhodtseva and their colleagues in the Soviet Union.

The interest in the reversible neuromodulation effects of ultrasound started to grow
in the 2000s with two in-vivo studies in mouse hippocampal slices [20, 19] and on
brain of mice ex-vivo [21], which proved the activation of action potentials after
ultrasound sonication. From that year, interest in this field gained momentum, as
indicated in the bottom part of Figure 2.1, where the number of papers published on
ultrasound neuromodulation up to 2008 and afterward are depicted. Starting from
2010, both excitatory and inhibitory effects were demonstrated in different areas
in rats and rabbit [19, 22, 4, 23, 24, 9, 25, 26, 27, 28, 29, 30]. The first study in
non-human primates is dated 2013, where a stimulus-locked response was observed
in two monkeys [31]. In the following two years, two research groups [2, 32, 1]
investigated the effect of US in the primary human somatosensory cortex, showing
both inhibitory [2, 32] and excitatory [1] effects. Interestingly, in 2015 Lee and
colleagues [1] employed software simulation to evaluate the effective intensity which
reached the brain. The effect of TFUS has also been investigated in combination
with other technologies, for example fMRI [23, 33], PET [3] or EEG [2, 32], and, more
recently, with TMS stimulation [34]. The interest in TFUS is not only restricted
to basic science, but its possible lasting effect [35] and therapeutic applications is
growing. For example, TFUS has been shown to help plasticity after stroke [6] and
to decrease epilepsy seizure [4].

It is worth noting, the different development of diagnostic ultrasound and ultrasound
for neuromodulation, as depicted in Figure 2.1. Starting from the 1940s [18], the
development of diagnostic ultrasound increased and never stopped, while for neuromodulation, the momentum was gained only in the last 10 years, and the interest in it is increasing.
Figure 2.1: A qualitative representation of the different number of papers published in diagnostic ultrasound (top) and in low intensity ultrasound modulation (bottom). The data are based on a search on Web of Science for papers including ‘diagnostic ultrasound’ (top) or ‘neuromodulation low intensity ultrasound’ (bottom) in the topic (March 2019). The number of papers on neuromodulation for the years 1920-2007 are based on the review [19].
CHAPTER 2. BACKGROUND

Figure 2.2: Acoustic waves can be of two types: a) longitudinal, in which the direction of wave propagation and the direction of movement of the particles is parallel; or b) transverse or shear wave, in which the direction of wave propagation is perpendicular to the direction of the movement of the particles.

2.3 Basics of acoustics

2.3.1 What is a wave?

An acoustic wave is a physical phenomenon during which mechanical energy is transferred through matter without mass transfer, and which originates from a local change in the stress or pressure field within the medium [36].

This change causes a local variation of pressure in the surrounding particles, that propagates into the medium over time. This leads to a creation of a periodic pattern in both time and space of low and high pressure, that can be described by the acoustic wave equation. The distance between two adjacent peaks or valleys in space is called the wavelength, indicated by $\lambda$, and the wavenumber, or spatial frequency, $k$, is defined as $k = 2\pi/\lambda$. At a fixed point in space, the number of peaks passing per second is called the frequency of the wave, indicated by $f$, and its reciprocal is called the period of the wave, $T$. The angular frequency, $\omega$, is $\omega = 2\pi f$. Ultrasound waves are a sub-group of acoustic waves with a frequency above the human audible range, that is above 20 KHz.

Waves can be transverse or shear, where the direction of wave propagation is perpendicular to the direction of the particle movement, or longitudinal, where the direction of wave propagation and the direction of movement of the particle is parallel (Figure 2.2). Shear waves require forces perpendicular to particles oscillation to transfer energy: therefore strong enough bonds are necessary. For this reason, transverse waves can only propagate in solids, and not in nonviscous fluid [36].

Biomedical applications of ultrasound involve mechanical waves in soft tissue, which
at a first and widely used approximation can be treated as fluid, and not as a solid. It means that mainly only longitudinal waves travel through such medium. It has to be noted that low-frequency shear waves can exist in soft tissue [36], but usually travel at low speed and are strongly absorbed [37].

Depending on the type of the wavefront, a wave can be classified as planar, when the wavefront is located on a plane that propagates in space, or circular, when it propagates symmetrically around a reference point (as a sphere or a ring), or around a reference line (as a cylinder) [36].

### 2.3.2 Acoustic variables

**Fluid particles and the continuum hypothesis**

A fluid element or particle is an abstract, imaginary infinitesimal volume of the fluid, that moves with the fluid and it is small enough that all variables are uniform throughout. Although small, the fluid element or particle is large enough to contain millions of molecules, so that the fluid can be thought of as a continuous medium (the continuum hypothesis) [38]. A medium is considered continuous in the sense that however small piece can be recursively divided by half, a single molecule would never be reached. This is of course not real, but it is a good approximation for length scales much larger than the average spacing between the molecules, which is of the order of nanometers in soft tissue. That means, as long as the distances are longer than a few hundred nanometers, the medium can be safely thought of as continuous [37].

**Particle displacement**

The particle displacement (Figure 2.3), indicated by the vector $\xi(x_0)$, is the displacement of a particle from its equilibrium position $x_0$ due to the local variation of pressure as the sound wave propagates. In the context of this thesis, it is assumed that the displacement is only caused by the acoustic wave.

**Particle velocity**

Assuming a null net flow velocity, the particle velocity $\mathbf{u}$ is equal to the acoustic velocity vector, defined for each particle as the time derivative of the particle displacement:
CHAPTER 2. BACKGROUND

Figure 2.3: The 1D particle displacement $\xi$, indicated by the green line, expresses the displacement of the particles with respect to their position at equilibrium, i.e. in an undisturbed medium. For simplicity, in this figure the particle displacement is shown along one spatial dimension ($x$). Adapted from [37].

\[ u = \frac{\partial \xi}{\partial t} \]  \hspace{1cm} (2.1)

The particle velocity must not be confused with the speed of sound.

**Pressure, density and temperature**

The acoustic pressure, density and temperature, can be expressed by the difference between their values at equilibrium and their instantaneous values (notation from [38]):

\[
\begin{align*}
\text{acoustic pressure} &= \mathcal{P} - \mathcal{P}_0 \\
\text{acoustic density} &= \rho - \rho_0 \\
\text{acoustic temperature} &= T - T_0
\end{align*}
\]  \hspace{1cm} (2.2)

where $\mathcal{P}, \rho, T$ are the instantaneous pressure, density, and temperature respectively, and $\mathcal{P}_0, \rho_0, T_0$ are the equilibrium pressure, density, and temperature, respectively. For the sake of simplicity, the acoustic pressure will be indicated as $p$. 

11
CHAPTER 2. BACKGROUND

2.3.3 Medium acoustic property

Mass density

The *density*, denoted by the greek letter $\rho$, of a particular homogeneous substance, is defined as its mass divided by its volume.

Compressibility

*Compressibility* is a measure of the change of volume of a certain substance when subjected to a difference in pressure. Under isothermal conditions, the coefficient of compressibility $\beta$ for a fluid is defined as

$$
\beta = -\frac{1}{V} \left( \frac{\Delta V}{\Delta P} \right)_T
$$

(2.3)

where $V$ is the original volume, $\Delta V$ the change in volume, $\Delta P$ the change in the pressure and the suffix $T$ indicates that the temperature is constant. Although fluids like water and oil are considered incompressible in many applications because $\Delta V$ is small even with large $\Delta P$, in the ultrasound context compressibility plays an important role and cannot be neglected.

Speed of sound

The *speed of sound* is the characteristic speed at which a sound wave travels in a given medium. Following this definition, the speed of sound $c$ can be written as

$$
c = f\lambda
$$

(2.4)

where $f$ is the frequency of the wave and $\lambda$ is the wavelength. The speed of sound in a given medium can also be written as a function of the compressibility $\beta$ and the density $\rho$ as

$$
c = \sqrt{\frac{1}{\beta \rho}}
$$

(2.5)

Further on, only the speed of sound at ambient conditions will be considered, and it will be indicated by $c_0$. 
Acoustic impedance

Similarly to electrical impedance, the *acoustic impedance* is a quantity that describes the opposition of a certain material to the acoustic flow given a certain pressure. It is indicated by the letter $Z$ and is the ratio between pressure and particle velocity. Since, for a plane wave, pressure can be expressed as $p = \rho_0 c_0 u$ then the acoustic impedance can be written as [36]

$$Z = \rho_0 c_0$$  \hfill (2.6)

For spherical waves, the expression for acoustic impedance is [36]

$$Z = \frac{\mu r \rho_0 (1 - ikr)}{1 + k^2 r^2}$$  \hfill (2.7)

where $i$ is the imaginary unit, $k$ is the wavenumber, and $r$ is the distance from the acoustic source. If $kr \gg 1$, then, using the equivalence $c_0 = \frac{\omega}{k}$, the impedance can be written as

$$Z = \frac{k \omega r^2 \rho_0}{k^2 r^2} = \rho_0 c_0$$  \hfill (2.8)

which is the same expression as for a planar wave. For TFUS application, supposing a wave frequency of 250 kHz and propagation in water with $c = 1500$ m/s, meaning a wavelength $\lambda = 6$ mm, and a distance from a point source of 3 cm, then $kr = r \frac{2\pi}{\lambda} = 30 \frac{2\pi}{6} = 31$. Therefore, since the condition $kr \gg 1$ is valid, the simplification can be made in TFUS applications.

2.3.4 Tissue/wave interaction

Reflection and refraction

A wave can pass through media with different speed of sound and density while propagating. Figure 2.4 depicts this situation: when a wave encounters the interface between the two media, a fraction of the wave is reflected, and a fraction is transmitted or refracted. It can be expressed by the *reflection coefficient*, $R$, and the *transmission coefficient*, $T$:
\[ R = \frac{p_r}{p_i} \]
\[ T = \frac{p_t}{p_i} \]  \hspace{1cm} (2.9)

where the subscripts \( i \), \( r \), and \( t \) stand for incident, reflected, and transmitted, respectively. Incident, reflected and transmitted waves must obey two **boundary conditions** [37]. The first states that the acoustic pressure must be the same on both side of the boundary because there should be no net force (continuity of pressure). Applying the first condition to Figure 2.4 leads to

\[ p_i + p_r = p_t \]  \hspace{1cm} (2.10)

The second condition states that the particle velocities normal to the boundary must be the same because the fluid must stay in contact (continuity of particle normal velocity). This leads to

\[ u_i \cos(\theta_i) + u_r \cos(\theta_r) = u_t \cos(\theta_t) \]  \hspace{1cm} (2.11)

where \( \theta_i \), \( \theta_r \), and \( \theta_t \) are the incident, reflected, and transmitted angles, respectively, as shown in Figure 2.4.

\[ \text{Figure 2.4: Two media with different speeds of sound and densities (} c_1, \rho_1 \text{ for the first medium and} c_2, \rho_2 \text{ for the second medium) are virtually separated by an interface. The incident wave, denoted by the subscript} i \text{ is partly reflected} (p_r) \text{ and partly transmitted in the other medium} (p_t). \text{The incident, reflected and transmitted wave angles} (\theta_i, \theta_r, \theta_t) \text{ are defined by taking the normal to the interface.} \]
CHAPTER 2. BACKGROUND

Dividing (2.10) by (2.11):

\[
\frac{p_i + p_r}{u_i \cos \theta_i + u_r \cos \theta_r} = \frac{p_i}{u_i \cos \theta_i}
\]  

(2.12)

And, since the acoustic impedance is \(p/u\), it can be written

\[
\frac{p_i}{u_i} = \rho_1 c_1 \quad \frac{p_r}{u_r} = -\rho_1 c_1 \quad \frac{p_t}{u_t} = \rho_2 c_2
\]  

(2.13)

where the minus arises because \(u_i\) and \(u_r\) have opposite directions. Considering this and that the angle of incidence is equal to the angle of reflection (\(\theta_i = \theta_r\)), equation (2.12) can be written as

\[
\frac{\rho_1 c_1 (p_i + p_r)}{\cos \theta_i (p_i - p_r)} = \frac{\rho_2 c_2}{\cos \theta_i}
\]  

(2.14)

That, after some rearrangement, leads to:

\[
R = \frac{\rho_2 c_2 \cos \theta_i - \rho_1 c_1 \cos \theta_i}{\rho_2 c_2 \cos \theta_i + \rho_1 c_1 \cos \theta_i}
\]  

(2.15)

From (2.10), \(T = R + 1\), so

\[
T = \frac{2 \rho_2 c_2 \cos \theta_i}{\rho_2 c_2 \cos \theta_i + \rho_1 c_1 \cos \theta_i}
\]  

(2.16)

When the incident wave is perpendicular to the surface, these indices become:

\[
R = \frac{\rho_2 c_2 - \rho_1 c_1}{\rho_2 c_2 + \rho_1 c_1} = \frac{Z_2 - Z_1}{Z_2 + Z_1}
\]

\[
T = \frac{2 \rho_2 c_2}{\rho_2 c_2 + \rho_1 c_1} = \frac{2Z_2}{Z_2 + Z_1}
\]  

(2.17)

where \(Z_1\) and \(Z_2\) are the acoustic impedance of the first and the second medium, respectively.

Finally, the square powers of the above indices give the ratio between reflected and incident intensity and transmitted and incident intensities. Since the intensity is proportional to \(\frac{p^2}{Z}\) (Supplementary Material in Appendix A) it can be written
\[ \frac{I_r}{I_i} = R^2 \]
\[ \frac{I_i}{I_i} = T^2 \frac{Z_1}{Z_2} \]  

(2.18)

Scattering

When a wave travels in a heterogeneous medium, the surface of the heterogeneities might reflect the wave. Depending on the size of the scatterers, different types of scattering can be defined [37]:

1. **Specular**: when the scatterers are much bigger than a wavelength, only strong reflections, as described in the previous section, occur.

2. **Diffractive**: If the obstacles have small openings or a length comparable with the wavelength (around 1 to 100 times), the wave tends to bend and spread around the edges. This phenomenon is called *diffraction* and allows the wave to propagate in zones where it would be otherwise shadowed.

3. **Diffusive**: when scatterers are much smaller than a wavelength the intensity of scattered wave is weak compared to the incident wave [37].

Attenuation and absorption

A wave amplitude decreases as it propagates, and this is called *attenuation*. This is due to several phenomena, like absorption (the conversion of kinetic energy delivered by the acoustic wave into heat), scattering, and reflection. It has been experimentally shown that the effect of absorption is dominating the effect of scattering [39].

The *absorption coefficient* is frequency-dependent and it can be written as

\[ \alpha = \alpha_0 f^b \]  

(2.19)

where \(\alpha\) is the absorption coefficient in units of Neper per meter (Np m\(^{-1}\)), \(f\) is the frequency, \(\alpha_0\) is a medium constant in Np m\(^{-1}\) Hz\(^{-b}\) and \(b\) is a numerical constant dependent on the tissue type. The absorption coefficient is often expressed in dB m\(^{-1}\). The relation between dB and Np is

\[ \alpha_{dB} = 0.115 \alpha_{Np} \]  

(2.20)
### 2.3.5 Wave equation

In this section, a brief overview of the derivation of the wave equation is given. A more detailed description can be found in [37, 38, 40].

Under the continuum hypothesis, the linear acoustic wave equation can be derived from three fundamental equations. A linear wave equation is sufficient because the deviations from equilibrium are small, meaning that the changes in velocity, density and pressure are small.

The first equation is the relationship between the particle velocity $u$ and the instantaneous density $\rho$ and expresses the medium compressibility (i.e. the density can change) [38]. Such relationship is called linear conservation of mass or linear continuity equation and needs

$$
\rho_0 \frac{\partial s}{\partial t} + \nabla \cdot (\rho u) = 0
$$

(2.21)

where $s$ is the condensation, defined as $s = \frac{\rho - \rho_0}{\rho_0}$. The second term in the left side can be written as:

$$
\nabla \cdot (\rho u) = \rho \nabla u + (\nabla \rho) u
$$

(2.22)

Under linear assumption, one has $s \ll 1$, meaning that $\rho \nabla u$ can be written as $\rho_0 \nabla u$. As shown in [40] (page 316), the term $(\nabla \rho) u$ is negligible, and therefore equation (2.21) can be expressed as

$$
\rho_0 \frac{\partial s}{\partial t} + \rho_0 \nabla u = 0
$$

(2.23)

The second equation represents Newton’s second law applied to a fluid element. The linearised momentum conservation equation or Euler’s equation is

$$
\frac{\partial u}{\partial t} = -\frac{\nabla p}{\rho_0}
$$

(2.24)

where $p = \mathcal{P} - \mathcal{P}_0$ (equation (2.2)).

The third equation is a relationship between the thermodynamic quantities and is expressed as a linear equation of state:

$$
p = c_0^2 \rho_0 s
$$

(2.25)
Differentiating (2.25) with respect to time gives:

\[
\frac{\partial p}{\partial t} = c_0^2 \rho_0 \frac{\partial s}{\partial t}
\]  

(2.26)

substituting in (2.23) gives:

\[
\frac{1}{\rho_0 c_0^2} \frac{\partial p}{\partial t} = -\nabla \cdot \mathbf{u}
\]  

(2.27)

differentiating this with respect to time gives

\[
\frac{1}{\rho_0 c_0^2} \frac{\partial^2 p}{\partial t^2} = -\frac{\partial}{\partial t} (\nabla \cdot \mathbf{u})
\]  

(2.28)

Differentiating (2.24) with respect to space, gives

\[-\nabla \cdot \left( \frac{\partial \mathbf{u}}{\partial t} \right) = \nabla \cdot \left( \frac{\nabla p}{\rho_0} \right)\]  

(2.29)

As \(\frac{\partial}{\partial t}(\nabla \cdot \mathbf{u}) = \nabla \cdot (\frac{\partial \mathbf{u}}{\partial t})\), the last two equations can be combined to give the linear wave equation for heterogeneous media

\[
\frac{1}{c_0^2} \frac{\partial^2 p}{\partial t^2} - \rho_0 \nabla \cdot \left( \frac{\nabla p}{\rho_0} \right) = 0
\]  

(2.30)

If the ambient density \(\rho_0\) is the same everywhere, equation (2.30) becomes the wave equation for homogeneous media

\[
\frac{1}{c_0^2} \frac{\partial^2 p}{\partial t^2} - \nabla^2 p = 0
\]  

(2.31)

This partial differential equation (PDE) has several weaknesses:

- as it discards high-order terms, non-linear propagation is not taken into account. However, non-linear phenomena are more prominent in high intensity focused ultrasound (HIFU) applications [39] and can therefore be neglected for LIFU.

- since the model is derived from fluid dynamics equations, the equation can only account for longitudinal waves, and not for shear waves. As already
mentioned, shear waves have a limited impact on fluids or soft tissues, but they can propagate in solids like bone. However, it has been shown that they are negligible when modeling the propagation of ultrasound beam through the skull for TFUS applications [41].

- energy absorption is not considered [39].

To include the attenuation in the equation, a term is added to (2.21)

\[ \rho_0 \frac{\partial s}{\partial t} = -\nabla \rho_0 \cdot \mathbf{u} - \frac{\tilde{a}}{c_0^2} p \] (2.32)

where \( \tilde{a} \) is equal to

\[ \tilde{a} = 2a \sqrt{\frac{a^2 c_0^4}{\omega^2} + c_0^2} \] (2.33)

The full calculation can be found in [39]. Substituting (2.26) in the previous equation, leads to

\[ \frac{1}{c_0^2} \frac{\partial p}{\partial t} = -\nabla \rho_0 \cdot \mathbf{u} - \frac{\tilde{a}}{c_0^2} p \] (2.34)

Differentiating the above equation with respect to time yields

\[ \frac{1}{c_0^2} \frac{\partial^2 p}{\partial t^2} = -\nabla \frac{\partial \rho_0}{\partial t} \cdot \mathbf{u} - \frac{\tilde{a}}{c_0^2} \frac{\partial p}{\partial t} \] (2.35)

Using (2.24) this equation can be written as

\[ \nabla^2 p - \frac{1}{c_0^2} \frac{\partial^2 p}{\partial t^2} - \frac{\tilde{a}}{c_0^2} \frac{\partial p}{\partial t} = 0 \] (2.36)

This equation is implemented in the acoustic solver of the simulation environment described in the next session.

### 2.4 Simulation environment

The acoustic solver of the simulation software *Sim4Life* (https://zmt.swiss/sim4life/) developed by IT’IS Foundation in Zürich has been used in this thesis with the aim
of validating the modeling framework to allow a more reliable dose control in TFUS experiment. In this section, the simulation environment will be briefly described. A more detailed description of the development of the Acoustic Solver and its features can be found in the PhD thesis [39].

2.4.1 Simulation methods

Many methods to simulate ultrasound fields are available, and they are less or more complicated depending on the complexity of the situation and the equations behind the modeling. For example, modeling of a round, single-element transducer in a lossless and homogeneous medium, is rather simple because it does not have to cope with attenuation, dispersion, and reflections and the symmetry of the problem reduces the number of spatial dimensions to two [42]. On the contrary, these simplifications do not apply to modeling of a phased array and a heterogeneous and attenuative medium like brain tissue and a more advanced method is required [42]. However, various approximations, e.g. associated with interest only in the area nearest to the ultrasound beam or discarting reflections, may be applied to reduce the numerical effort [42]. Under restricting assumptions, for example medium homogeneity, mathematical expressions can explicitly explain the acoustic field generated by a transducer. These expressions are integrals of analytical functions and therefore qualify as being semi-analytic [42]. These models are fast, simple and easy to implement numerically, but they are not usable for complex scenarios like brain stimulation, due to the underlying restricting assumptions. For this reason, these situations require numerical methods for solving the partial differential equations describing the pressure field.

2.4.2 Finite-differences time-domain methods

A finite-difference time-domain method (FDTD) is a numerical method to solve PDE equations. It solves the equation in the time domain (TD), and not in the frequency domain, by using local basic equations and replacing the mathematical differentiations by numerical finite differences (FD) [42].

The different steps of this method and how they are implemented in the acoustic solver employed in this thesis are presented in the following sections. The description is adapted from [39].
CHAPTER 2. BACKGROUND

Geometrical discretization

The first step of the method is to divide the simulation model (a set of 2D or 3D geometries) into small parts, in a process called discretization. In this process, the setup is gridded into grid cells, and together they form a rectangular or rectilinear grid, called the computational grid. Computational grids can be uniform when the grid cells all have the same dimensions, or nonuniform when grid cells have different dimensions. The latter allows for a more flexible discretization of complex geometries.

After discretization, each grid cell is assigned a single material type, typically based on which part of the setup occupies the majority of the grid cell volume. This process is called voxeling, and the resulting set of rectangles (2D geometries) or cuboids (3D geometries) are called voxels.

Stencil derivation

After the discretization process, calculation of the field quantities is based on the central finite-differences formulas, which are derived from Taylor series expansion.

Let’s consider a function $F$, which in our case is the acoustic pressure $p$, whose values are spatially and temporally dependent. Assuming that the grid cell locations on a uniform grid are defined by a set of $(i, j, k)$ indices, the first-order central finite difference formulas for the spatial derivatives are:

\[
\frac{\partial F}{\partial x}(i\Delta x, j\Delta y, k\Delta z, n\Delta t) = \frac{F^n_{i+1,j,k} - F^n_{i-1,j,k}}{2\Delta x} \\
\frac{\partial F}{\partial y}(i\Delta x, j\Delta y, k\Delta z, n\Delta t) = \frac{F^n_{i,j+1,k} - F^n_{i,j-1,k}}{2\Delta y} \\
\frac{\partial F}{\partial z}(i\Delta x, j\Delta y, k\Delta z, n\Delta t) = \frac{F^n_{i,j,k+1} - F^n_{i,j,k-1}}{2\Delta z}
\]

(2.37)

Similarly, the second-order central finite difference formulas for spatial derivatives are
Lastly, assuming that the temporal instants of the calculated field are denoted by an index \( n \), the first and second order temporal derivatives can be calculated with the following formulas:

\[
\frac{\partial F}{\partial t} (i \Delta x, j \Delta y, k \Delta z, n \Delta t) = \frac{F_{i,j,k}^{n+1} - F_{i,j,k}^{n-1}}{2 \Delta t}
\]
\[
\frac{\partial^2 F}{\partial t^2} (i \Delta x, j \Delta y, k \Delta z, n \Delta t) = \frac{F_{i,j,k}^{n+1} - 2F_{i,j,k}^{n} + F_{i,j,k}^{n-1}}{\Delta t^2}
\]

(2.39)

Equations (2.37) through (2.39) can be used to decompose full-wave PDEs, such as (2.36) into a series of simple arithmetic operations, thus allowing for their straightforward numerical implementation. Such equations are typically called computational stencils. For a full description of their derivation see [39].

As it can be clearly seen in equations (2.37) through (2.39), an essential point is the choice of the space and time step in the simulation environment. To improve the accuracy of the simulations, the grid size must be chosen using this well-establish rule of thumb

\[
\Delta_{\text{max}} \leq \frac{\lambda_{\text{min}}}{10}
\]

(2.40)

where \( \Delta_{\text{max}} \) is the maximum grid step and \( \lambda_{\text{min}} \) is the minimum wavelength in the entire computation domain.

To ensure the numerical stability, the following condition for the time step needs to be true:

\[
\Delta t \leq \frac{1}{c_{\text{max}} \sqrt{\frac{1}{\Delta x_{\text{min}}^2} + \frac{1}{\Delta y_{\text{min}}^2} + \frac{1}{\Delta z_{\text{min}}^2}}}
\]

(2.41)

where \( c_{\text{max}} \) is the maximum speed of sound and \( \Delta x_{\text{min}} \), \( \Delta y_{\text{min}} \) and \( \Delta z_{\text{min}} \) are the
minimum cell widths along their respective axes in the entire domain.

**Boundary model**

The simulated space cannot be infinite, so the domain needs to be truncated. However, it should model a real situation, where the domain is open. To obtain this, an absorbing boundary condition (ABC) on each of the domain boundaries is applied. ABCs allow for the numerical absorption of propagating waves to create the illusion of an infinitely extending boundary.

In particular, in the software used in this thesis, a perfectly matched layer (PML) ABC was used. Briefly, in the PML implementation, multiple layers of artificial absorbing material are placed adjacent to the boundaries of the computational grid. The entire computational domain is then surrounded by a perfectly reflecting boundary. The artificial material is matched to the surrounding material to avoid reflection at the interface. Therefore, the outgoing waves entering this material are attenuated and they decay exponentially. The returning reflecting waves are negligibly small. A detailed explanation of its implementation can be found in [39].

**Source modeling**

In the used simulation environment, the acoustic source is modeled using Huygen’s principle. This principle states that every point on a wave-front is itself a source of spherical waves. Given that, the piezoelectric acoustic source can be included in the computational domain as any other object and then voxelized into a large number of source-voxels. During simulations, each of these source-voxels generates spherical acoustic waves with a given pressure amplitude, frequency, and phase. When they are driven with identical properties, the resulting wave is equivalent to that of a non-discretized piezoelectric element. This discretization allows for advanced source-modeling capabilities, such as modeling a source with nonuniform pressure distribution.
The previous sections emphasized the necessity of establishing the TFUS *therapeutic window* to expand its applications from initial piloting studies to broader testing in humans.

The manuscript presented in Appendix A explains in details how to calculate the safety indices used in diagnostic ultrasound. Moreover, it collects information on the following topics, based on the available literature of both human and animal experiments:

- What are the main possible harmful effects of ultrasound on tissues? What are the causes and how can they be assessed?

- What are the main recorded transient and lasting neural and behavioral effects of TFUS and what are the possible causes?

- For each study, the observed neural effect and the parameters used are stated and related to the safety indices used for diagnosis and therapy.

The review presented in Appendix A shows that the application of TFUS in animals and humans was safe in most (31/33) of the screened studies. In two animal studies where a long sonication or a sonication at high intensity for a short time was applied, the ex-vivo analysis of the brain shows signs of microhemorrhages. However, since these studies didn’t thoroughly investigate the possibility that TFUS was not the cause of the microhemorrhages, for example by adding sham conditions, these findings should be confirmed in future studies. In two studies a high-temperature increase (> 2.5° C) was estimated in the skull or measured in the beam after long sonication (40 s or more). This means that an assessment of heating should be
performed as a standard procedure when establishing new protocols that exceed the FDA safety limits for diagnostic US.
CHAPTER
FOUR

MEASUREMENTS FOR THE VALIDATION OF A
MODELING FRAMEWORK FOR REALISTIC
SCENARIOS

The previous chapter presented a common setup for TFUS (Figure 2 of Appendix A). In this project, we have replicated part of the same arrangement to characterize the ultrasound beam in a pure water background, without and with obstacles. The description of the setup, the analysis of the data and the performed characterizations will follow in the next sections. The performed measurements are included in the manuscripts in Appendices B, C and D. The following additional information, however, is not part of these Appendices.

4.1 Setup for measurements in a pure water background

Figure 4.1 shows a schematic of the employed setup. It consists of three parts: the first generates the pressure wave with the same characteristics shown in Figure 2 in Appendix A. In particular, a transducer receives a voltage signal and converts it into a pressure wave, due to its piezoelectric element. This wave propagates into the medium and when it reaches the hydrophone, the latter turns the received pressure into a voltage signal. The second part of the system collects, visualizes, and stores the data from the hydrophone. The third part of the setup moves the hydrophone in the medium to reconstruct pressure or intensity maps from distributed points in
Chapter 4. Measurements for the Validation of a Modeling Framework for Realistic Scenarios

Both the transducer and the hydrophone are in a tank filled with deionized water. A more detailed description of these parts will follow.

Signal generation

A function generator creates square waves with a certain amplitude ($A_t$), duty cycle (DC), repeated every pulse repetition period (PRP). A second function generator produces a burst of a certain duration -named tone burst duration (TBD)-, at a given center frequency ($f_c$), with a specific amplitude (A). The burst is triggered by the rise of the signal from the first function generator to create a train of bursts of duration TBD repeated every PRP. The amplitude A is then amplified by a power amplifier with a gain G, given in dB, corresponding to a voltage amplification by a factor of $10^{G/20} \text{dB}$. The amplified train of bursts drives the transducer. Figure 4.2 shows that the measured voltage depends linearly on the voltage applied to the transducer.

Stepper-motor control and hydrophone holder

Figure 4.3 depicts the water tank and the screw stepper motors employed in Denmark. Matlab scripts and functions written by ourselves send commands to three screw stepper motors, oriented in three perpendicular directions (x-, y-, and z-axis). The motors move the hydrophone holder to acquire data from the desired volume. Briefly, the user can set the initial and the final position of the target plane, together with the sampling distance (the distance of the red dots in Figure 4.1). When the script starts, it runs executable files that send commands to the motor via a USB connection.

Since the objects in the water tank might reflect the wave, we tried to reduce this effect by putting a sponge around the hydrophone aluminum holder. In a second try, we changed the material of the holder, substituting aluminum with Plexiglas, which has an acoustic impedance similar to water. Moreover, the transducer holder has two oblique faces, preventing the reflected waves to be directed to the hydrophone. However, while reflection has a big impact when continuous waves are employed, the application of sufficiently short pulsed waves makes its effect negligible.

Acquisition of data and storage

A USB cable connects the oscilloscope to the computer, and the same script used to move the motor can store data from each point through VISA (Virtual Instrument Standard Architecture) and SCPI-commands (Standard Commands for Pro-
Figure 4.1: The setup for measurements in a water tank. **Signal generator**: An amplified train of bursts, generated by 2 function generators, drives the US transducer. **Control of stepper-motor**: 3 stepper-motors (not indicated) move the hydrophone to acquire the pressure wave in transverse (blue) and parallel (orange) planes relative to the transducer. **Data acquisition**: an oscilloscope receives and sends the voltage signals from the hydrophone to the computer where they are stored. A square wave synchronizes the generation and the acquisition of the pressure.
Figure 4.2: The measured voltage has a linear relationship with the peak-to-peak voltage ($V_{pp}$) applied to the transducer. In fact, on average, the measured peak-to-peak voltage is 0.4 times the applied voltage.

grammable Instruments-commands). Since the wave is pulsed, the square wave from the first function generator triggers the acquisition. To reduce the noise, we used the average mode of the oscilloscope, which averages 32 signals in time at each measurement position.

Data Analysis

The stored raw signals were first filtered with a high pass 4th order Butterworth filter with a cutoff frequency of 200 kHz to remove low-frequency noise (for pressure waves with a center frequency of 500 kHz). An example of the signal before and after the application of the filter is shown in Figure 4.4.

For each measured position, the intensity was then calculated as

$$I = \frac{p^2}{2\rho c} \quad (4.1)$$

where $\rho$ is the density of water ($1000 \ kg/m^3$) and $c$ is the speed of sound in water ($1500 \ m/s$). The pressure $p$ was found taking the mean peak-to-peak voltage amplitude in the steady state (from the 5th to the last pulse of each burst) and dividing it by the hydrophone sensitivity $ML$ at the center frequency, as indicated by the
4.2 Performed beam characterizations

As mentioned in the Introduction, dosing control is challenging in in-vivo human studies due to the inter- and intra-variability of skull anatomy and composition, which makes it difficult to predict the intensity after cranial transmission based on measurements in a pure water background. For this reason, the beam characterizations, without and with obstacles, described in this chapter were used to validate a modeling framework for a single element focused transducer (SEFT). Appendix B
and C focused on the development of an accurate model for the transducer which accounts for the actual physics and geometry of the transducer. Table 4.1 shows the values of the parameters used, and a detailed description on the performed acquisition and how the objects (Figure 4.7) were fixed in the water tank can be found in Appendix B. Not all of the acquired data were included in the study because the impact of the object was negligible (see Figure 4.8 for lamb skull) or too far from a realistic situation (3D printed sphere, in Figure 4.7).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Used value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_c$</td>
<td>500 KHz</td>
</tr>
<tr>
<td>A</td>
<td>$K_R$: 600 mV</td>
</tr>
<tr>
<td></td>
<td>$D_K$: 10 mV</td>
</tr>
<tr>
<td>TBD/T</td>
<td>20</td>
</tr>
<tr>
<td>PRP</td>
<td>1 KHz</td>
</tr>
<tr>
<td>$A_t$</td>
<td>$K_R$: 2 Vpp</td>
</tr>
<tr>
<td></td>
<td>$D_K$: 3.3 Vpp</td>
</tr>
<tr>
<td>DC</td>
<td>50 %</td>
</tr>
</tbody>
</table>

Table 4.1: The parameters used for the water tank measurement. Some values used in Denmark (indicated by $D_K$) differ from the ones used in Korea (indicated by $K_R$). The characterization of the transducer in water and the data set with the skulls were performed in Korea, while the data set with the obstacles printed in VeroBlack was acquired in Denmark.

We also focused on the mapping of acoustic properties of skulls from CT data to
CHAPTER 4. MEASUREMENTS FOR THE VALIDATION OF A MODELING FRAMEWORK FOR REALISTIC SCENARIOS

Figure 4.5: Example maps of intensity, normalized to the maximum.

Figure 4.6: An example intensity map (top) in water background in units of $mW/cm^2$ and the relative phase map in radians (bottom).

take into account tissue inhomogeneities. This work is presented in Appendix D.
CHAPTER 4. MEASUREMENTS FOR THE VALIDATION OF A MODELING FRAMEWORK FOR REALISTIC SCENARIOS

Figure 4.7: Some of the obstacles placed between the transducer and the hydrophone. A) the fragment of the pig skull (left), the sheep skull (center), and the lamb skull (right). B) two of the 3D printed objects in VeroBlack material: the (100x100x5 mm$^3$) rectangular volume (left) and a sphere with a diameter of 2 cm (right).

Figure 4.8: Example intensity maps without and with the presence of skulls. The intensities are normalized to the maximum in water.
CHAPTER

FIVE

CMUT AND MAGNETIC RESONANCE COMPATIBILITY

This chapter presents the characterization of the intensity beam and initial work on the MRI-compatibility of a capacitive micromachined ultrasonic transducer (CMUT) ring array with an outer diameter of 8.1 mm and an inner diameter of 5.2 mm. The main advantage in the use of CMUT over the usual piezoelectric transducer is the possibility to miniaturize the device to establish TFUS in freely-moving animals, allowing more tests, for example on the lasting effects after prolonged stimulation without anesthesia. A detailed description of the process of fabrication, the setup and a general review on the CMUT technology is beyond the scope of this thesis and can be found in [43] and Appendix E. Briefly, a superposition of an AC voltage, generated as indicated in Figure 4.1, over a DC voltage from a power supply, drive each element of the array, resulting in ultrasonic pressure waves with an amplitude which depends on the AC voltage. The CMUT array lies on a printed circuit board (PCB), and the cables delivering the ground and the driving signals are soldered directly to the PCB, as shown in Figure 5.1.

A first test to verify the effect of a CMUT array and its parts on a clinical 3T scanner forms part of the paper presented in Appendix E.

In this work, we first tested the MRI-compatibility of the CMUT array itself, and then added the different parts needed to deliver the voltages to the system. Specifically, we tested how the static magnetic field $B_0$ in the MRI bore and an EPI signal, which is usually employed to acquire the BOLD signal in fMRI, were affected by the presence of the device. We showed that the device did not affect the MR magnetic field nor the recordings, even when it was powered up. In addition, we tested
Figure 5.1: The CMUT array and the PCB board where it lies used in this part of the thesis. A 2 kroner coin, with a diameter of 2.45 cm, is shown on the right.

whether the device induced RF noise. When the device was connected to the amplifier and DC power source (both outside the MR cabin), RF noise was transported into the scanner by the cables. This problem can be solved, for example, by passing the currents through a low pass filter attached to the MR cabin.
CHAPTER SIX

CONCLUSIONS AND FUTURE PERSPECTIVES

6.1 Safety of TFUS

The first aim of the project was to summarize the available information and data regarding the possible harmful effects of TFUS to establish the upper limit of the therapeutic window. This work has been presented in Chapter 3 and Appendix A. TFUS was found to be safe in the majority of the screened studies. However, we highlighted the necessity of further investigations to demonstrate the reproducibility of the observed outcomes, in particular when signs of microhemorrhage were observed. Further investigations of the therapeutic window of TFUS are needed. In particular, knowing how long stimulation can be performed without irreversible consequences is indispensable to expand the clinical applications of TFUS, allowing safe sustained exposure to induce long-lasting effects. Employing higher intensities might also be decisive for obtaining more robust neural responses of TFUS, together with more reliable studies of its acute effects and their causes. Moreover, our review highlighted the need for specific examinations of the thermal bio-effects on the skull and brain induced by TFUS at different intensities and duration to establish the complete safety profile of TFUS.

To achieve this goal, an estimation of the temperature increase due to the sonication through the bio-heat equation might be achieved by simulating the precise pressure profile which is delivered to the brain during the whole stimulation duration. Moreover, simulations can help in the estimation of the possible unwanted secondary foci due to standing waves inside the skull cavity. These standing waves might result in an actual intensity at the primary or secondary foci higher than the estimated one.
from measurements in a water tank with a fragment of a skull. Overall, our study on safety highlighted the need for a more specific guideline for the use of TFUS in humans. This new guideline will probably be different from the standards for the diagnostic and therapeutic application of ultrasound, and will probably require characterization of the beam, the possible second foci, and temperature increase after cranial transmission.

### 6.2 Dose control

The previous section highlights the importance of a reliable procedure for dose control, i.e. the control of the intensity at the target after skull transmission. Moreover, the dose should be estimated before each experiment to allow a more robust response, decrease the variability of the dose across subjects and make outcomes from different studies better comparable. To implement an accurate dose control, we have used a software simulation framework. As initial step, we focused on how to model the transducer, investigating the effect of modeling the details of the internal structure, rather than fitting the transmitting surface to match the hydrophone measurements (Chapter 4 and Appendices B and C). Our results showed that simulation results are more accurate when the entire internal structure of the transducer and not only the external effective surface, that is a surface with changed geometry to fit the measurements, is modeled, especially when obstacles are present. Our results gave an insight into how the acoustic properties of the medium where the wave is propagating can change the estimated beam profile. The speed of sound mainly changes the beam shape, while attenuation affects the portion of the beam which is transmitted through the skull. For a reliable dose control with simulations, therefore, both attenuation and speed of sound should be properly modeled.

Extending these initial steps towards an accurate dose control, we are currently working on a detailed investigation of the mapping of acoustic properties of the skull from CT data. As shown in Appendix D, a model of the skull which takes into account inhomogeneity will improve the fit with real data. CT scans employ X-ray to image the body and different radiations doses, measured in Sievert (Sv), can be used. For clinical applications, the dose is usually 1-2 mSv. As we are not interested on resolving details within the skull cavity, we can substantially decrease the radiation dose. Based on experience from another project, acceptable CT scans of the skull can be achieved for doses of $\sim 0.3$ mSv on a modern clinical CT scanner. The higher the dose, the higher is the risk for the scanned person to develop cancer (even though the risk is very low already for 1-2 mSv). On the other hand, the higher the dose, the better SNR (signal-to-noise ratio) the resulting image will have. As an example, Figure 6.1(A, B) compares two images, one at dose for research
purposes (namely ‘low dose’), and one with a dose much bigger than the clinical dose (namely ‘high dose’, used as reference). In order to reduce the level of noise, different reconstruction filters can be set during CT data acquisition. For example, smooth filters can reduce the level of noise of the image, but they will, however, decrease the level of details in the image (see Figure 6.1 C, D). In addition, the quality of the CT data strongly depends on the background where the object is. For example, Figure 6.1(E, F) shows the CT data of the same object (the pig skull sample), with the same dose and filter, in water and air background. The latter has a better SNR. The CT images of the human head have an SNR in between these two because the brain absorbs X-rays similarly to water. Therefore, we are currently testing the dependence of the simulated TFUS beam after skull transmission to find the best compromise between image smoothness and SNR that allows for accurate simulations of the beam at clinical and low CT doses.

6.3 CMUT-MRI compatibility

Our aim is to establish a setup that allows to image the neural effect induced by TFUS with fMRI online in rodents. Following our promising results on the combination of a CMUT array in a clinical MRI scanner presented in Chapter 5 and Appendix E, we started more extensive tests to combine the CMUT device presented in the mentioned sections of the thesis in a high field (7T) preclinical scanner. The transducer array lies on a printed circuit board (PCB) (Figure 5.1), and the driving signal with the ground signal is applied to the transducer array via cables and two metal rings, which surround the array and are connected with every single element of the array by very thin bonding wires. Our preliminary results suggest that the presence of the packaged CMUT causes drop in echo-planar imaging (EPI) signal quality, as shown in Figure 6.2, and introduce field inhomogeneities (Figure 6.3). Part of this effect is due to the radio frequency (RF) noise induced by the cables: in fact, the quality of the images improved when the cables were removed (Figure 6.2 and 6.3). The use of a low pass filter might help in filtering out the RF noise from the cable. However, the cause of the remaining disturbance of EPI signals and B0 field should be investigated. To achieve this aim, a systematic testing of each component can help, in the sense that disturbances due to the PCB or the transducer array should be assessed separately. For example, the presence of two metal rings on the PCB might cause eddy currents when the gradients are applied. After the debugging, a re-design of the packaged device might be required.

In parallel to testing the CMUT transducer in the preclinical MRI, I set up hydrophone measurements of its beam profile to be able to assess its full functioning in the time course of testing and later applications in rodents. I therefore replicated
Figure 6.1: Different examples of how CT parameters and the type of background (water vs. air) can change the SNR of the images of a fragment of a pig skull. A high dose image (A) has a better SNR respect to the same image at a low dose (B). In particular, the higher level of noise is visible in both the background and the pig skull sample. The images (A, B) have a sharp filter and the objects are in air background. The use of a sharp reconstruction filter (C) leads to an image with more details in it, together with more noise. On the contrary, a smoother filter leads to less noise, but losses some details (D). For example, the details in the central bottom part of the skull in (C) are almost no more visible in (D). Both images (C, D) have high dose and are in air background. Finally, an air background (E) will result in much higher SNR, while a water background will result in lower SNR (F). Both images (E, F) are at high dose and with sharp filter.
CHAPTER 6. CONCLUSIONS AND FUTURE PERSPECTIVES

Figure 6.2: The mean of 16 EPIs images with PCB, CMUT, and cables, and with PCB and CMUT but without cables. The relative reference images acquired without PCB, CMUT, and cables are also shown. The device is on the top of the phantom, and it clearly reduces the signal compared to the reference image. Part of the reason can be RF noise from cables because when they are removed, the signal is higher.

the setup presented in Figure S3 in Appendix E. Our setup is shown in Figure 6.4, which is based on the setup described in Chapter 4, and used it to perform the beam characterization of the device shown in Figure 5.1. In particular, I used a smaller tank filled with rapeseed oil, and printed a specific holder for the CMUT device with a 3D printer. Figure 6.5 shows a comparison between the beam measured in the original setup (Figure 1-F in Appendix E) and our measurement. The measurements fit well well with those obtained at KAIST, even though the acquired plane was not chosen big enough to sample the entire beam.

In summary, starting with the CMUT transducer presented in Chapter 5 and Appendix E, we are currently progressing in establishing a complete setup required for in-vivo MRI in rodents. We aim to use this setup for systematic testing of the safety profile and neural mechanisms of TFUS.

6.4 Future work

A correct evaluation of the therapeutic window of TFUS needs an accurate control of dose at the target. Moreover, estimation of the dose before the experiment is essential to stay within the therapeutic window, once established. My PhD thesis was aimed at validating and improving a simulation framework to estimate the acoustic dose after skull transmission. However, investigations on temperature increases and standing waves were not performed. Measuring the beam reflections inside a skull
CHAPTER 6. CONCLUSIONS AND FUTURE PERSPECTIVES

Figure 6.3: The magnitude (left) and the B0 field map (right) with PCB, CMUT, and cables (B), and with PCB and CMUT but without cables (D). The reference images acquired without the CMUT in place are also shown (A, C). The device is on the top of the phantom, and it clearly reduces the magnitude and induces B0 inhomogeneities compared to the reference image. Part of the reason can be RF noise from cables because when they are removed, the signal is slightly higher and the B0 field is less affected.

Figure 6.4: This figure shows the adapted setup to acquire the beam profile of the CMUT in a rapeseed oil background. A specific holder for the CMUT was designed and 3D-printed.
Figure 6.5: An example of the intensity maps of CMUT under rapeseed oil. On the top (A), the intensity maps from the original setup (Figure 1F in Appendix E) and in the bottom our measurement. The plane transverse to the CMUT is in the left column, while on the right the parallel plane (corresponding to the purple line on the left) is shown. The intensity is specified in units of dB. The measurements fit well, but the acquired plane was not big enough to sample the entire beam, and therefore we need further measurements to sample the beam closer to the device to make a proper comparison.
might be challenging with the measurement in water tank described in Chapter 4, because the hydrophone cannot be inserted and freely moved inside the skull cavity. In this sense, MR studies employing ex-vivo skulls might help when the temperature increase is high enough to be picked up by MRI thermography.

Complementary to the aim to further validate the simulation framework, a specific safety study in rodents that aims to replicate and extend the findings of microhemorrhages for longer or more intense parameter settings as discussed in Appendix A might be valuable. The possibility to apply TFUS during in-vivo MRI in rodents will allow us to measure the brain response to TFUS using BOLD fMRI (Blood Oxygen Level Dependent functional MRI). By that, the spatial position at which neural effects occurred can be systematically compared with the positions of adverse effects. In addition, fMRI can be used to establish the lowest dose at which TFUS induces neural activity, as required to establish the therapeutic window. BOLD fMRI might also serve to some extent as a control for simulations of the TFUS beam inside the rodent skull cavity, as the small dimensions will result in complex standing wave patterns at the frequencies that are used for human TFUS. In order to be able to transfer findings about safety from rodents to humans, low frequencies might be still preferable for this study despite a limited possibility of spatial focusing, so that the characterization of the spatial intensity distribution in the skull cavity can be challenging.

Our long-term aim is establish the necessary procedures and knowledge to perform safe TFUS studies in humans. An accurate and reliable procedure to control the dose does not only benefit safety, but will also result in more robust results with minimized differences between interindividual doses and a higher probability of replication of the results among different research centers. At least in near future, reliable dose control will still require water tank measurements and the possibility to acquire calibrated CT images in addition to performing the computer simulations. This makes the procedure relatively complex and requires expert knowledge. However, without accurate dosing, TFUS intensity has to be chosen very conservatively in humans, which increases the likelihood of underdosing. Therefore, I think that TFUS research will strongly benefit from accurate dose control.


Appendices
SAFETY OF TRANSCRANIAL FOCUSED ULTRASOUND STIMULATION: A SYSTEMATIC REVIEW OF THE STATE OF KNOWLEDGE FROM BOTH HUMAN AND ANIMAL STUDIES

The following manuscript has been submitted to Brain Stimulation and is currently under revision.
Safety of transcranial focused ultrasound stimulation: 
A systematic review of the state of knowledge from both human and animal studies

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Abstract
Background: Low-intensity transcranial focused ultrasound stimulation (TFUS) holds great promise as a highly focal technique for transcranial stimulation even for deep brain areas. Yet, knowledge about the safety of this novel technique is still limited.

Objective: To systematically review safety related aspects of TFUS. The review covers the mechanisms-of-action by which TFUS may cause adverse effects and the available data on the possible occurrence of such effects in animal and human studies.

Methods: Initial screening used key term searches in PubMed and bioRxiv, and a review of the literature lists of relevant papers. We included only studies where safety assessment was performed, and this results in 33 studies, both in humans and animals.

Results: Adverse effects of TFUS were very rare. At high stimulation intensity and/or rate, TFUS may cause haemorrhage, cell death or damage, and unintentional blood-brain barrier (BBB) opening. TFUS may also unintentionally affect long-term neural activity and behaviour. A variety of methods was used mainly in rodents to evaluate these adverse effects, including tissue staining, magnetic resonance imaging, temperature measurements and monitoring of neural activity and behaviour. In 30 studies, adverse effects were absent, even though at least one Food and Drug Administration (FDA) safety index was frequently exceeded. Two studies reported microhaemorrhages after long or relatively intense stimulation above safety limits. Another study reported BBB opening and neuronal damage in a control condition, which intentionally and substantially exceeded the safety limits.

Conclusion: Most studies point towards a favourable safety profile of TFUS. Further investigations are warranted to establish a solid safety framework for the therapeutic window of TFUS to reliably avoid adverse effects while ensuring neural effectiveness. The comparability across studies should be improved by a more standardized reporting of TFUS parameters.
Keywords: transcranial focused ultrasound, TFUS, safety, histology, review
Introduction

Weak Transcranial Focused Ultrasound Stimulation (TFUS) aims to modulate neural activity by delivering a focused ultrasonic beam to a small target area in the brain. Currently, interest in TFUS is strongly increasing as it holds the promise of a far better spatial resolution than established non-invasive stimulation techniques and of the ability to reach deep brain areas [1]. This might open up intriguing new applications such as epilepsy treatment or pre-surgical diagnostics prior to electrode implantation for deep-brain stimulation [2, 3]. TFUS is also attractive because it can be readily combined with neuroimaging modalities such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) without interfering with the recordings, as it applies acoustic waves rather than electric or magnetic fields.

Firmly establishing its safety profile is a central requirement when aiming to move TFUS from initial pilot studies towards broader testing in humans in-vivo. Reviews on safety and bio-effects of ultrasound (US) in diagnostics [4] and therapy [5] as well as guidelines for the clearance of commercial diagnostic and therapeutic US systems as medical devices [6] are available and constitute a benchmark to avoid harmful effects also for TFUS. Relating the TFUS parameters to these guidelines, as done in many of the published studies, might be considered a conservative choice. However, several aspects put TFUS in a special position. TFUS usually employs lower frequency compared to diagnostic ultrasound (usually upper kHz range vs. MHz) and longer pulse bursts. TFUS has a static focus so that the total energy delivered at the focal point can be higher than the maximal local energy deposit for diagnostic US, as the latter uses scanning approaches. The mechanism-of-action of TFUS is still poorly understood, rendering it more difficult to principally exclude harmful effects. In addition, current findings about the dose-response curve of TFUS [7] suggest that future therapeutic applications might aim to use intensities above the safety limits for diagnostic US in order to increase the robustness of the neural effects. Such a choice requires solid knowledge about the safety margin of TFUS. Along similar lines, accurate dose control for human TFUS is complicated by the presence of the skull, which strongly attenuates the beam. The attenuation depends on the individual skull thickness and composition [8], which are difficult to account for and lead to
conservative intensity choices with an increased risk of underdosing. If the safety margin of TFUS is not well established, the use of more lenient dosing strategies to mitigate this problem is not feasible.

There is a pressing need to establish specific safety guidelines for TFUS. Yet, the current knowledge about the risk-benefit ratio and the therapeutic window of TFUS is still rudimentary because TFUS is at an early stage of development. Indeed, no dedicated phase I safety human study has been performed so far, but the safety profile needs to be systematically investigated and monitored to ensure the patients’ safety. However, relevant information is already available today, because some of the published studies on TFUS in animals or humans included safety-relevant tests. Here, we systematically summarize these findings to give an overview of the current state of knowledge about TFUS safety. We start by describing the relevant physical parameters used to characterize the TFUS stimulus. We then shortly describe the known physical mechanisms by which ultrasound can cause tissue damage and we introduce the established safety indices, based on the beam parameters. Finally, we introduce the methods that have so far been applied to test for adverse effects of TFUS, and list the corresponding results. In the discussion, we summarize the implications of the available findings for in-vivo human TFUS applications.

**Material and Methods**

*Literature review on the safety of TFUS*

For this systematic review, we followed the PRISMA guidelines [9, 10]. Details on the implementation of the PRISMA requirements in our review are stated in the Supplementary material (Table S1). Our review was based on searches in PubMed (www.ncbi.nlm.nih.gov/pubmed) and bioRxiv (https://www.biorxiv.org/) for published and pre-published studies, using the keywords ‘tFUS’, ‘LIFUP’, ‘noninvasive brain stimulation focused ultrasound’, ‘neuromodulation brain transcranial ultrasound’, ‘focused ultrasound transcranial brain stimulation’ and ‘pulsed ultrasound brain stimulation’. The eligibility criteria were low intensity, low
frequency TFUS in the brain of animals or humans with safety assessment, without use of microbubbles. Additional sources were reviews of the literature lists of relevant papers, and papers pointed out by the reviewers during the peer-review process. Figure 1 shows details of the literature search. The last complete search was performed in January 2019 by one of the authors, and the last update was done in June 2019. From each paper, the sonication parameters and the methods used to assess safety and adverse effects were extracted as shown in Table 2 and Table 3 and categorized as described further below. Often, only some of the safety indices were reported. In that case, we give estimated values when possible.

**Mechanism of ultrasound neuromodulation**

Despite many hypotheses, the exact underlying mechanism of neuromodulation using low-intensity ultrasound is yet to be understood [11]. The initial hypotheses for the ultrasound neuromodulation were thermal effects and acoustic cavitation. While an increase in the tissue temperature could perturb neuronal activity levels, the temperature increase due to low-intensity ultrasound is often less than 0.1°C. Thus, the thermal effects of low-intensity ultrasound are most likely negligible. The second hypothesis is based on acoustic cavitation. This hypothesis postulates that the ultrasound generates nanobubbles in the lipophilic zone of the plasma membrane, which then vibrates according to the pressure variations, alters the local curvature of the bilayer, and changes overall neuronal excitability [12]. However, since nanobubbles are formed at an intensity larger than 100 mW/cm², generation of micro or nanobubbles at the intensity used in standard neuromodulation protocols must be confirmed. The recent hypotheses now focus more on the effects of acoustic radiation forces on the permeability of the ion channels, such as mechanosensitive channels [13] and voltage-gated calcium, sodium, and potassium channels [14]. Another kind of hypotheses includes plasma deformation, which postulates that vibration of surrounding extra- and intracellular environment evokes mechanical changes in either the plasma membrane tension or the lipid bilayer and modulates neuronal activities [14].
Contrary to these works on the mechanisms involved with direct modulation of ion channels and membranes, an indirect in vivo ultrasound neuromodulation through auditory or cochlear pathways has been also recently proposed [15, 16]. These studies demonstrated that ultrasound-induced activities were eliminated or reduced upon transection of the auditory nerves or removal of cochlear fluids. These results raised an important question of whether direct activation of neurons in the intact brain is possible. While more in-depth studies on the experimental protocols such as sharpness of the pulse, pulse repetition frequency, and bone transduction must be performed, these studies underscore the need for a solid understanding of the underlying mechanism of ultrasound neuromodulation [16].

Physical parameters and safety indices of US waves

A sketch of an experimental setup for TFUS is shown in Figure 2A, using the stimulation of a rat as example. The main indices used to assess safety are:

- $I_{\text{spTa}}$ (spatial peak temporal average intensity) is the temporal average intensity, calculated at the position of the spatial maximum
- $I_{\text{spPa}}$ (spatial peak pulse average intensity) is the pulse average intensity, calculated at the position of the spatial maximum
- $MI$ (mechanical index) gives an estimation of the likelihood of inertial cavitation
- $TI$ (thermal index) is the steady-state temperature increase in soft tissue during ultrasound sonication
- $TIC$ (thermal index for cranial bone) is a modification of $TI$, when the skull is close to the transducer face

$I_{\text{spTa}}$, $TI$ and $TIC$ are related to the risk of thermal bio-effects, while $I_{\text{spPa}}$ and $MI$ are related to the risk of cavitation. The upper limits for these five indices allowed for diagnostic ultrasound are shown in Table 1. It should be noted that another guideline, IEC standard 60601-2-5 for physiotherapy US equipment, sets an
upper limit for the “effective intensity”, defined as the ratio of acoustic output power to effective radiating area, of 3 W/cm². The standard also states that this value should only be reached for short times to prevent substantial heating. The “effective intensity” of 3 W/cm² is usually interpreted as the upper limit for $I_{\text{pta}}$ [17, 18, 19]. Lee and colleagues [17] compare the intensities used in their study against this limit rather than using the FDA guidelines for diagnostic US. Complementary to TI, the temperature increase at the target can be calculated as $\Delta T_{\text{max}}$ (equation 7 and 8 in Supplementary material) or through the bio-heat equation [20, 21, 22]. A more detailed explanation of these indices and formulae can be found in the Supplementary Material.

**Mechanisms underlying tissue damage by US**

Ultrasound waves may cause harmful effects on tissues via two physical mechanisms, mechanical and thermal. The main mechanical effect is cavitation, in which vapor cavities (or “bubbles”) form in the soft tissues during the periods of low pressure (i.e. the minima) of the acoustic wave cycles. Depending on intensity and center frequency, this can result in a stable oscillation (stable or non-inertial cavitation) or can result in violent bubble collapses (inertial cavitation) that create large forces in their neighborhood. The air bubbles can have an endogenous origin (for example in the lungs or intestine), or they can be created by the mechanical wave itself, if the peak rarefaction pressure (i.e. the pressure during the minima) is small enough to allow the liquid to reach vaporization. Alternatively, ultrasound contrast agents (UCA), which contain microbubbles, can be injected for, e.g. clinical purposes [23] or gene and drug delivery [24].

When a mechanical wave propagates linearly in a medium, its amplitude decreases exponentially starting from the source. The attenuation is caused by both scattering, i.e. the change in the direction of wave propagation due to the presence of microscopic obstacles along the beam, and absorption. Absorption is the process by which the wave energy is converted into heat, and therefore the medium is heated. Several ways to model or monitor the resulting temperature increase in the medium exist, and they will be further discussed below.
Types of adverse and side effects caused by TFUS

In this section, we summarize the potential adverse and side effects, which have so far been tested in TFUS studies, and briefly outline the employed techniques to assess the occurrence of these effects. The majority of results were obtained in animal studies, which tested for the following effects:

- **Blood-brain barrier (BBB) opening**: The BBB is a semi-permeable membrane formed by endothelial cells which separates the vessels and the central nervous system (CNS) [25]. Air bubbles subjected to cavitation can break the BBB. Exploiting this effect, TFUS combined with US contrast agents is tested as a method for targeted drug delivery [26]. However, BBB opening is undesired for normal TFUS. Assessing BBB integrity is usually based on the intravenous injection of a substance, which cannot cross the barrier under normal conditions, prior to sonication. It is then tested whether TFUS causes the substance to diffuse into brain tissue. The dyes fluorescein isothiocyanate-dextran (FITC-dextran) [27], trypan blue [28, 29, 30] or Evans blue [31] have been used for this purpose, and their presence inside the brain was investigated in post-mortem microscopy analyses of brain slices. Alternatively, an MRI contrast agent (a gadolinium chelate) was injected before the stimulation and its penetration into brain tissue was tested by assessing the MRI signal change due to the contrast agent [29].

- **Bleeding**: The occurrence of bleeding has been investigated using tissue staining, in particular hematoxylin and eosin (H&E) staining [28, 32, 33, 2, 19, 34, 35, 36, 37, 38, 22], which reliably stain blood cells. Yet, H&E staining is not specific to blood cells and thus requires experience to correctly interpret the results.

- **Cell death and damage**: A general approach to qualitatively analyze the presence of cell death and damage is through H&E staining [28, 2, 34, 29, 35, 32, 30, 39, 33, 21, 19] [36, 37, 38, 40, 22], as described above, cresyl violet Nissl staining [22], or luxol fast blue dye (LFB) [21], used to identify
myelin in nervous tissue. Cell death can be of two types, apoptosis and necrosis. While apoptosis is part of the normal life cycle of the cells, necrosis is harmful and triggered by external factors or disease. It is possible to differentiate between both types of cell death based on morphological criteria, but this requires experience [41]. Additional techniques specifically label apoptotic cells and have therefore been used to distinguish between apoptosis and necrosis. For example, the presence of fragmented DNA is a sign of apoptosis, and not necrosis, and it can be labeled by terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay [29, 2]. Alternatively, since apoptosis is mediated by caspase [42], standard immunocytochemistry techniques with antibodies against cleaved caspase-3 can be used [27, 30].

An alternative approach to detect cell damage is staining for acidophilic cells, for example with VAF (vanadium acid fuchsin) [30, 43]. Acidophilia refers to the property of cells of staining readily with an acid dye and occurs after acute neuronal damage and death in brain ischemia.

Finally, also transmission electron microscopy has been used to quantitatively observe the effect of ultrasound on brain ultrastructure (postsynaptic density, docked vesicles, etc.) [27]. It has been shown that neural trauma causes an abnormal increase in the number of astrocytes [44] that can be detected by the expression of GFAP (glial fibrillary acidic protein) [31, 30, 39]. One study assessed possible permanent tissue damage after sonication in rats using MRI [39].

*Irreversible changes of neural activity:* Recordings after the sonication can determine whether changes in neural activity are reversible and characterize the duration of recovery. The effects on local neural activity in the TFUS target region can be detected directly via invasive recordings or voltage sensitive dyes [31] or Ca²⁺ imaging in transgenic mice that express the green fluorescent calcium indicator [31]. TFUS-related changes in extracellular concentrations of excitatory and inhibitory neurotransmitters such as glutamate and λ-aminobutyric acid (GABA) can be measured via microdialysis techniques [37]. TFUS has also been combined with measurements of the forelimb and hindlimb responses to epidural cortical stimulation (ECS) to assess the cortical excitability changes after sonication [45]. Alternatively,
electroencephalography (EEG) [29], functional MRI [22, 46], PET [32], measurements of peripheral muscle evoked potentials (MEP) [47], sensory evoked potentials (SEP) [48], somatosensory evoked potentials (SSEP) [21], visual evoked potentials (VEP) [49] or auditory evoked potentials (AEP) [39] give non-invasive but less specific measurements of neural activity changes.

- **Undesired changes in animal behavior:** TFUS may affect normal behavior in unintended ways. In animals, this is controlled by monitoring of everyday behavior, like food uptake, defecation and movement behavior and checking for signals of pain and distress or change in weight [28, 34, 29, 32, 30, 33, 45, 50]. In addition, tasks such as the rotorod task and wire-hanging task allow for quantitatively assessing the impact of TFUS on specific aspects of behavior [27]. One study induced ischemic stroke in mice and compared the behavioral changes of the mice which were treated with TFUS via a balance test and an adhesive removal test [19].

Adverse effects can be caused by cavitation or tissue heating. As outlined above, cavitation is prevented by controlling the pressure levels. While the temperature increase in the brain can be roughly estimated using Equations (7) and (8) in the Supplimentary Material [19, 27, 51, 35, 17, 34, 30, 48], some studies inserted a thermocouple [27, 28, 48, 33, 45] or an optical fiber based thermal sensor [36] in the brain of the animal after craniotomy to track the temperature change in real time during sonication. A non-invasive alternative to this approach is measuring the temperature increase with thermocouples in a phantom [33], or MR thermometry [29, 52, 39, 21], which exploits temperature sensitive MR parameters such as the water proton resonance frequency, or T1 and T2 relaxation times [53].

So far, most TFUS studies used animal models. Tests for adverse effects in the few human studies were based on neurological examinations and/or structural MR imaging before and at one or several time points after the experiment [51, 17, 54]. In some of the studies, the participants were additionally contacted by telephone 2 months after the experiment and interviewed about any changes in their mental and physical health status, including experiences of any discomfort [51, 17]. A pre-print manuscript [55] presents results
of phone interviews based on a ‘Participant report of symptoms questionnaire’ of 64 participants who had participated in one or more of seven human TFUS experiments before.

Results

Studies screened in this review

This systematic review follows PRISMA guidelines [10, 9], and the PRISMA checklist can be found in the Supplementary Methods (Table S1). The reviewing process shown in the PRISMA diagram Flow (Figure 1) resulted in the selection of 31 peer-reviewed and 2 pre-published studies included in this review. From each of those papers (a complete list with citations is shown in Table 2), the sonication parameters (Table 2) and the methods used to assess safety and adverse effects (Table 3) were extracted and categorized as described further below. Often, only some of the safety indices were reported. In that case, we give estimated values when possible. The risk of bias was assessed and is reported in a separate section in the Supplementary Material.

BBB opening

BBB opening did not occur in any of the included studies [28, 29, 30], except for two cases where it was intentionally provoked in control conditions [31, 27], using a high $l_{sppa}$ of 280 W/cm$^2$ [31] or an ultrasound contrast agent [27].

Bleeding

Several studies [28, 32, 33, 2, 19, 36, 37, 38, 22] tested for bleeding, without finding evidence for it. A further study [34] tested different sonication parameters on eight sheep in total, and reported four animals with micro-hemorrhages in the primary visual cortex after undergoing 600 sonications at 6.6 W/cm$^2$ $l_{sppa}$ (6 repetitions of 100 sonications, with 30 s gaps). While the reported value for $l_{sppa}$ is within FDA limits, our calculated value for $l_{spta}$ of 3.3 W/cm$^2$ is exceeding the diagnostic limit, and is also slightly higher than the...
limit for physiotherapeutic US of 3 W/cm². Interestingly, a sheep undergoing a single sonication at an Isppa of 13.4 W/cm² did not present micro-hemorrhages. In another study [35], 1 of 37 rats was exposed to a high intensity (11.2 W/cm² Ispta) for a short period of time (< 9 s using 1 ms TBD, 50% duty cycle and 300 ms SD). It exhibited several areas containing hemosiderin, which indicate the potential of local bleeding, while none of the other animals showed any sign of bleeding.

**Cell damage or death**

Most of the studies testing for cell damage or death [28, 2, 34, 29, 35, 32, 27, 19, 30, 39] [33, 21, 36, 37, 38, 40, 22] did not observe harmful effects of TFUS. One recent study [31] observed no differential GFAP expression between the control and sonicated hemisphere for Isppa = 0.69 W/cm², suggesting the absence of neural trauma. However, an increased number of astrocytes was observed for a control condition with Isppa = 280 W/cm² (~ 1.5 times above the FDA limit). Interestingly, no damage was observed even when AEP was not fully recovered after one month [39] in rats.

**Long-term change of neural activity**

Yoo et al [29] tested parameter ranges for excitatory and inhibitory TFUS effects in craniotomized rats. While excitatory effects were very short-termed, suppression effects lasted several minutes. A reduction in the EEG response of up to 80% and a corresponding reduction of the BOLD signal that both lasted up to 10 minutes were reported for a long sonication duration of 9 s. Dallapiazza et al. [21] observed peak electrophysiologival suppression in SSEP 5 minutes post-treatment, and the values returned to near baseline within 20 minutes. A further study [31] tested the facilitatory effects of ultrasound on somatosensory evoked potentials by measuring the changes in fractional fluorescence in the brains of mice dyed with voltage sensitive dyes. The TFUS-related changes disappeared within 20 minutes after ultrasound stimulation. Yang et al. [37] observed a decreased extracellular GABA level (approximately 20% below baseline) compared to a control group that lasted up to 100 minutes after the sonication ended. The same effect was not observed for glutamate. Gulick et al. [45] showed that TFUS significantly suppressed forelimb
and hindlimb responses to ECS for several minutes after the stimulation blocks, even though effects immediately after single, short TFUS trials were absent. Kim et al. [32] observed a local increase in glucose metabolism induced by FUS to rat brain. This effect was demonstrated via PET imaging, which was started 20 minutes after the sonication and performed for 1 hour. After that time, the metabolism had still not returned to baseline. In a work [22] on primates, the authors observed change in functional connectivity after a long sonication of 40 s at \( I_{\text{sppta}} = 7 \, \text{W/cm}^2 \). The change lasted for more than one hour after sonication. A similar effect was observed in a related work [46], where they used a sonication of 40 s at a maximum \( I_{\text{sppta}} \) of 15.3 \( \text{W/cm}^2 \). Yoo et al. [48] observed that the SEP signals after 10 minutes sonication were distinctively different compared to the control condition, even 35 min after the sonication. Daniels et al. [39] observed a full recovery of AEP amplitudes in rats within maximum 1 week post-treatment with \( I_{\text{sppa}} = 2.3 \, \text{W/cm}^2 \), while the signal from 5 out of 10 rats recovered up to one month post-treatment for an \( I_{\text{sppa}} = 4.6 \, \text{W/cm}^2 \). In the same study, 1 out of 5 pigs showed a fully recovered signal 1 hour post-treatment while the other did not show any recovery 3 hours post-treatment (in all 5 cases, \( I_{\text{sppa}} = 4.6 \, \text{W/cm}^2 \)). Kim et al. [49] observed an increase in VEP in rats up to 5 minutes post-treatment with \( I_{\text{sppa}} = 5 \, \text{W/cm}^2 \) and a slight increase of VEP 150 s after treatment when \( I_{\text{sppa}} = 3 \, \text{W/cm}^2 \). One study [19] induced ischemic stroke in mice and found a better sensorimotor performance in mice that underwent 20 minutes TFUS session via a balance test and an adhesive removal test. These improvements lasted for 4 weeks after treatment, suggesting an enhancement in brain plasticity. In the same study, the TFUS treatment in cerebellar LCN significantly lowered the percentage change in increased water content and tissue swelling in the ipsilateral hemisphere to the stroke.

_Article behavior_

Several studies tested for changes from normal daily behavior after the sonication studies [28, 34, 29, 32, 30, 33, 45, 50], but did not find any abnormalities. A single study also employed behavioral tasks [29] (rotorod running task and wire-hanging task), without revealing differences in motor performance.
Temperature

Theoretical calculations based on Equations (7) and (8) in the Supplementary Methods suggest that “typical” TFUS parameters used so far in most studies cause negligible temperature increases in brain tissue [19, 27, 51, 35, 17, 34, 30, 48]. In a recent study [22], this was partly confirmed using the more realistic bioheat equation to estimate the temperature increase after 40 s of TFUS through a 3 mm thick skull, with an $I_{spta}=7 \text{ W/cm}^2$ in the brain. The maximal increase in the brain was less than 0.2°C. Interestingly, however, they found rather strong increases in the skull (2.8°C). Also experimental results show mostly only small temperature increases due to sonication [27, 28, 48, 33, 36]. However, it is important to note that the overall temperature increase depends on the combination of several TFUS parameters. For example, one study [36] reported a measured peak temperature increase of 0.2°C for an extended stimulation (~30 min) at a low $I_{spta}=230 \text{ mW/cm}^2$ at 1 MHz (1.6°C at 5 MHz for otherwise same parameters). In contrast, another study [45] reported a temperature increase up to 3°C after two blocks of 5 minutes stimulation at 200 kHz, separated by a 2 minutes break, at $I_{spta} = 4.5 \text{ W/cm}^2$ and a MI=3.1 (higher than the allowed limit). Both studies applied longer durations than used in most other TFUS studies so far, but the combination with the higher $I_{spta}$ caused noticeable temperature rises in the second study.

A temperature increase of 0.5°C was reported through MR thermometry after 30 s sonication at $I_{sppa} = 9.9 \text{ W/cm}^2$ [52]. Another study [39] reported temperature variation within the measurement noise level of the baseline temperatures (±2°C) with MR thermography. The strongest effect was reported by a study using MR thermometry (sensitivity 0.3±0.06°C [29]), demonstrating an increase of ~0.7°C in the sonicated area [29], using an $I_{sppa}=23 \text{W/cm}^2$ for 27 s. Dallapiazza et al. [21] showed a negligible temperature increase during treatment using both MR thermometry and estimations based on the bio-heat equation.

Findings from human studies

In a recent preprint work [47], the authors tested the effects of ultrasound stimulation on motor cortex excitability measured by single-pulse transcranial magnetic stimulation (TMS). They report significant
changes in the recorded muscle responses to TMS only when it was applied during, but not after, sonication. Follow-up neurological exams and anatomical MRIs after the TFUS experiment did not reveal any abnormalities or changes in the mental or physical status, nor any discomfort associated with the procedure [51, 54, 17]. Follow-up interviews at later time points confirmed those observations. A recent study published as preprint [55] presents results from a follow-up questionnaire after TFUS that could be obtained from 64 out of in total 120 participants. Seven subjects reported mild or moderate symptoms (mild neck pain, scalp tingling, headache, difficulty paying attention, muscle twitches and anxiety) that they felt were possibly or probably related to the experiment. These initial symptoms disappeared upon follow-up. The authors found a linear correlation \( r=0.797, \ p=0.0319 \) between \( I_{\text{ssa}} \) and the occurrence of observed symptoms among the 7 subjects who reported mild to moderate symptoms that were perceived as ‘possibly’ or ‘probably’ related to participation in TFUS experiments.

**Discussion and Conclusions**

Harmful effects of TFUS were absent in the majority of the 33 studies reviewed here. In two cases, microhemorrhages occurred in a subset of the tested animals when using a high \( I_{\text{spta}} \) of 11.2 W/cm\(^2\) for a short duration [35] or an \( I_{\text{spta}} \) of \( \geq 3.3 \) W/cm\(^2\) for a high number of sonications \((\geq500)\) given at a relatively short ISI of 1s [34]. Both doses are clearly above the safety limits of the FDA guidelines for diagnostic US and above the IEC standard 60601-2-5 for physiotherapy US equipment. However, this also holds for several other included studies, where no adverse effects were reported. While the parameters chosen in one of the studies [35] did not result in substantial heating, as also pointed out by the authors, the high \( I_{\text{spta}} \) of 11.2 W/cm\(^2\) differentiates it from many other TFUS studies. That might indicate that mechanical effects caused the microhemorrhages, even though the limits for MI and \( I_{\text{ssa}} \) were not exceeded. However, as this was observed in only one of the tested animals, this conclusion remains very speculative and a replication including sham controls would be favorable to ensure that the microhemorrhages were indeed related to
TFUS. In the second study [34], the chosen parameter combination might have led to a high total energy deposit, opening the possibility that a thermal mechanism underlay the adverse effects that occurred in four animals. For example, Gulick et al. [45] observed a temperature increase of 3°C for a less intense protocol using an $I_{spta} = 4.5 \text{ W/cm}^2$ and in total 180 sonications in a time period of 13 minutes. It seems reasonable to assume that heating might have been even higher in the four animals that showed microhemorrhages in [34] and indicates that calculating the temperature increase for a single sonication, as done in [34], can strongly underestimate the real increase.

While $I_{sppa}$ stayed below the safety limit in all studies, MI exceeded the limit in two studies [45, 46] and $I_{spta}$ exceeded the FDA limits for diagnostic US in soft tissue in 10 out of the 15 studies in which $I_{spta}$ was reported or could be calculated post hoc (values after cranial transmission or for craniotomized animals). $I_{spta}$ was also above the physiotherapeutic limit in 7 of the 15 studies. This suggests that $I_{spta}$ is the most sensitive safety index in case of TFUS and, unlike current practice, should be reported so that it can be followed up by a more detailed estimation of the thermal effects when its limits are exceeded. We consider this relevant as the current studies indicate that TFUS parameters within the FDA limits for diagnostic US might often lack neural stimulation effectiveness. For example, an $I_{spta}$ of around 2 $\text{W/cm}^2$ for pulsed waves and 4 $\text{W/cm}^2$ for continuous waves was necessary to reach a 50% success rate for stimulation at 500 kHz [7]. Similarly, while many studies included in this review reported neural effects for parameters within the safety limits [31, 27, 56, 48, 52, 33, 49, 19, 2], several studies found stable effects only when exceeding at least one of the safety indices ( [28, 34, 29, 35, 30] and Table 2). In addition, recent studies show that heating of the skull (potentially causing indirect heating of soft tissue) and/or brain tissue can reach several degrees for more intense and long protocols [22] [45]. The systematic assessment of heating will thus be relevant in future studies that might aim at extending the parameter envelope of TFUS and should be part of any safety test of new sonication regimes in particular for human TFUS.
It is worth noting that the safety limit of 720 mW/cm² for $I_{spt}$, which was generally used in TFUS studies so far and which we also applied here, was introduced to limit the heating in soft tissue. In case of transcranial US, the FDA limits for diagnostic US actually apply an even stricter limit of 94 mW/cm² for $I_{spt}$ to prevent excessive heating of the skull, which absorbs most of the beam energy. It seems that almost none of the studies published so far reported neural effects for intensities below this threshold. However, it is important to stress that both limits are based on worst-case scenarios and exceeding them does not necessarily mean that strong heating occurs. Rather, the FDA standard for diagnostic US requires a case-by-case estimation of the maximum temperature rise in soft tissue and skull once they are exceeded, specific for the used ultrasound parameters and setup. Simulations of the propagations of the TFUS beam through the skull, combined with evaluations of the bio-heat equation for TFUS [22], might be valuable tools that allow realistic estimates of the amount of heating for new sonication regimes on a more standard basis.

The neural aftereffects can exceed one hour [22, 32, 46], making TFUS a potent neuromodulation modality. This is encouraging for therapeutic applications. In contrast to diagnostic US, future TFUS applications might resort to repeated sessions over extended time periods to achieve and maintain therapeutic efficacy. As such, a safety framework will also need to cover these more intense settings (see, e.g. [57, 58] for a related example of adverse effects that only occurred after repeated applications in case of transcranial direct current stimulation) or combinations of TFUS with other brain stimulation techniques. This will require safety studies that specifically test this parameter space in order to inform an international consensus on accepted settings and procedures, similar to established non-invasive brain stimulation methods [59]. Along similar lines, in the few TFUS studies performed in humans so far, the type and extent of follow up exams differed strongly [55, 51, 54, 17]. This suggests a need for guidelines that provide a secure framework for experimental settings and practical procedures, including mandatory safety screening and appropriate follow-up procedures. For example, the importance of establishing best practices also for apparently simple procedures was highlighted in a recent review [60] of low-intensity low-frequency US
(20-100 kHz), showing that US can cause skin damage due to inertial effect cavitation in the coupling gel if non-degassed gel is employed.

Along similar lines, guidelines are important to prevent intensity hotspots that can occur due to unintended standing waves and focusing effects of the skull. While these effects more likely emerge in small animals [50], they have been shown to be also relevant in non-human primates for targets close to the skull base such as the amygdala [46]. Finally, the reviewed studies differed in regards to the choice of the stated safety-relevant parameters and the way those were assessed. A more standardized reporting of the relevant pulse parameters and of all safety indices of the FDA guidelines is a prerequisite for the development of future TFUS guidelines for human applications. Accurate estimation of the TFUS intensity after cranial transmission is particularly challenging in humans, as it has to rely on hydrophone measurements based on “representative” skull samples or computer simulations [61, 51, 17]. The uncertainty range of the intensity estimates obtained by these procedures seems still unclear [62, 63], and contributes to variations in the values reported across studies. As such, it seems useful that future studies additionally state intensity values for a pure water background to ensure good comparability of the baseline TFUS parameters.

Conflicts of interest
None declared.

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Bibliography


FIGURES AND TABLES

Figure 1: Selection process for the studies included in this review. The scheme is from [10, 9].
Figure 2: Overview of the TFUS setup and parameters.  A) The ultrasound pressure wave is generated by a transducer and delivered to the target through a guide filled with acoustic gel. B) The pressure stimulus over time is shown to indicate the main parameters. C) The main intensity values are shown for a fixed space position, together with their relationship with the pressure signal.

\[ I(t) = \frac{p(t)^2}{\rho c} \]

- \( I_{ta} \) = temporal average intensity
- \( I_{pa} \) = pulse average intensity

If these values are calculated in the position of the spatial maximum, they are called:

- \( I_{spta} \) (spatial peak temporal average intensity)
- \( I_{sppa} \) (spatial peak pulse average intensity)

\( ISI = \) inter stimulation interval
\( SD = \) sonication duration
\( TBD = \) tone burst duration
\( PRP = \) pulse repetition period (1/PRP = PRF, pulse repetition frequency)

**Duty cycle** = TBD / PRP
<table>
<thead>
<tr>
<th>$I_{\text{spta}}$ (mW/cm$^2$)</th>
<th>$I_{\text{sppa}}$ (W/cm$^2$)</th>
<th>MI</th>
<th>TI</th>
</tr>
</thead>
<tbody>
<tr>
<td>720</td>
<td>190</td>
<td>1.9</td>
<td>6</td>
</tr>
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</table>

Table 1: Allowed limits for MI, TI, $I_{\text{spta}}$ and $I_{\text{sppa}}$ according to the FDA guidelines for diagnostic ultrasound. The limit for TI also applies to TIC when bone is close by.
<table>
<thead>
<tr>
<th>Study</th>
<th>Target</th>
<th>Parameters</th>
<th>Observed neural effect and adverse effect (if any)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legon et al.</td>
<td>Human thalamus or M1</td>
<td>Follow-up questionnaire of 7 experiments, only 3 are published so far ([61, 64] and one preprint [47])</td>
<td>This work presents results on safety assessment.</td>
</tr>
<tr>
<td>Verhagen et al. [22]</td>
<td>Non-human primate SMA, FPC and pre-SMA</td>
<td>250 Hz 30 ms 10 Hz 40 s 1 - 2.4 in water * 1.68 after cranial transmission (estimated from pressure peak) * 48 W/cm² in water * 23.52 W/cm² after cranial tx * 14.4 W/cm² in water * 7.056 W/cm² after cranial tx *</td>
<td>Reversible change in brain connectivity, that last up to 2 hours after treatment.</td>
</tr>
<tr>
<td>Fisher et al. 2018 [31]</td>
<td>Mice primary somatosensory cortex</td>
<td>510 µs 500 µs 1 kHz 1 s 1 - 0.24 in water * 0.69 W/cm² in water 345 mW/cm² in water *</td>
<td>Early sensory-evoked cortical responses (3.0 ± 0.7 ms earlier) and alteration of Ca²⁺ responses.</td>
</tr>
<tr>
<td>Tufail et al. 2010 [27]</td>
<td>Mouse motor cortex</td>
<td>500 µs 0.45 ms 1.5 kHz 67° (53)° 180° 10 s 0.13 after cranial tx 211.72 mW/cm² after cranial tx * 142.1 mW/cm² after cranial tx</td>
<td>Neuron’s spike frequency and c-fos’ cell density increase and the activity of endogenous brain-derived neurotrophic factor (BDNF) were stimulated. Low frequency (250 KHz) and low intensities (up to around Iₛₚₚₐ=80 W/cm²) result in more robust EMG response. The EMG failure probability increased with shorter ISI (200 ms), but decrease with multiple stimuli. BBB intentionally opened with the use of microbubbles.</td>
</tr>
<tr>
<td>Kim et al. 2012 [28]</td>
<td>Rat abducens nerve</td>
<td>350 µs 0.96 ms 1.5 kHz 200 ms 10 1 s 0.9 after cranial tx (estimated) 8.6 W/cm² after cranial tx (estimated) 4.6 W/cm² after cranial tx (estimated)</td>
<td>t=650 kHz and Iₛₚₚₐ in the range 0.6-20 W/cm² did not elicit eye movement in any animals. Movements observed</td>
</tr>
</tbody>
</table>
when $f_c = 350$ KHz for an $\text{Isppa}$ of $8.6$ W/cm².

Lee et al. 2015 [34]

<table>
<thead>
<tr>
<th>Sheep SM1 and V1</th>
<th>250</th>
<th>1 ms</th>
<th>500 Hz</th>
<th>300 ms</th>
<th>100 (groups of sonications repeated up to 8 times per animal)</th>
<th>in the range 0.5-1.4 after cranial tx – SM1</th>
<th>Up to 11.8 W/cm² after cranial tx – SM1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up to 14.3 W/cm² after cranial tx - V1</td>
<td>Up to 7.15 W/cm² after cranial tx - V1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Up to 5.9 W/cm² * after cranial tx - V1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MEP or VEPs were detected over a certain intensity threshold, which varied across sheep and was always above diagnostic limits, and in some cases also above the physiotherapy limit. In both cases, higher $\text{Isppa}$ result in stronger response amplitude. Four animals which underwent 600 sonications at $\text{Isppa} = 6.6$ - $10.5$ W/cm² showed microhemorrhages in the primary visual cortex.

Yoo et al. 2011 [29]

<table>
<thead>
<tr>
<th>Rabbit (after craniotomy), SM and visual area (the bottom line is only for temperature increase study)</th>
<th>690</th>
<th>0.05, 0.5, 10, and 50 ms</th>
<th>10, 20, 100, and 1000 Hz</th>
<th>0.5, 1, 1.5, 2, 9 s</th>
<th>&lt;0.5 in water (for an $\text{I}_{\text{ppa}}=3.3$ W/cm², resulting in clear BOLD activity)</th>
<th>3.3, 6.4, 9.5, 12.6 W/cm² in water</th>
<th>1.6 W/cm² in water (for $\text{I}_{\text{ppa}}=3.3$ W/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td>The BOLD activation was observed at a much lower acoustic intensity ($\text{I}<em>{\text{ppa}}=3.3$ W/cm², $\text{I}</em>{\text{pta}}=1.6$ W/cm²) compared to the intensity that resulted in forepaw movement ($\text{I}<em>{\text{ppa}}=12.6$ W/cm², $\text{I}</em>{\text{pta}}=6.3$ W/cm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>690</td>
<td>0.5 ms</td>
<td>100 Hz</td>
<td>27 s</td>
<td>1</td>
<td>-</td>
<td>7</td>
</tr>
</tbody>
</table>

Parameter tested as control for temperature increase.

Lee et al. 2015 [51]

<table>
<thead>
<tr>
<th>Human S1</th>
<th>250</th>
<th>1 ms</th>
<th>500 Hz*</th>
<th>300 ms</th>
<th>Around 200</th>
<th>3 s</th>
<th>0.62 after cranial tx (maximal simulated value across N=12 subjects)</th>
<th>3 W/cm² in water</th>
<th>2.5 W/cm² after cranial tx (maximal simulated value)</th>
<th>1.5 W/cm² in water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td>Tactile sensations were not the same among subjects, but mostly at the hand area contralateral to the sonicated hemisphere. 1 out of 12 subjects did not report any sensation. Different peak amplitudes of EEG recording of SEP with and without stimulation.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lee et al. 2016 [54]

<table>
<thead>
<tr>
<th>Human S1+S2</th>
<th>210</th>
<th>1 ms</th>
<th>500 Hz</th>
<th>300 ms</th>
<th>20</th>
<th>7 s</th>
<th>35 W/cm² in water</th>
<th>&lt; 8.8 W/cm² after cranial tx (estimated)</th>
<th>17.5 W/cm² in water</th>
<th>&lt; 4.4 W/cm² after cranial tx (estimated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Response rates of elicited sensations during the FUS procedures were different among subjects (88±28% S1, 59±22% S2, 61±26% S1+S2, average±sd across subjects).</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Author et al. 2014 [35] | Rats | Somatomotor area | 350 and 650 | 0.25, 0.5, 1, 2, 3 or 5 ms | in the range [0.06, 2.8] kHz and continuous wave | 150, 200, 300 or 400 ms | 7 | 2 or 3 s | 1.38 (value for animal with signs of bleeding) | 22.4 W/cm² after cranial tx (max value reported, corresponding to animal with signs of bleeding) | 11.2 W/cm² after cranial tx (max value reported) | Motor responses were observed at observed at minimum threshold \( I_{\text{min}} = 4.9 \text{ to } 5.6 \text{ W/cm}^2 \), \( I_{\text{opt}} = 2.5 \text{ to } 2.8 \text{ W/cm}^2 \) in a limited range of sonication parameters (TBS=1-5 ms, 50% of duty cycle, and SD=300 ms, at fc=350 kHz). Pulsed sonication elicited motor responses at lower acoustic intensities than its equivalent continuous sonication \( I_{\text{opt}}=7.73 \text{ W/cm}^2 \). One animal which underwent a sonication of \( I_{\text{opt}}=11.2 \text{ W/cm}^2 \) for a short period of time (< 9 s using 1 ms TBD, 50% duty cycle and 300 ms SD) showed signs of local bleeding.

| Author et al. 2013 [32] | Rats | 350 | 0.5 ms | 1 kHz | 300 ms | 1200 * | 2 s | 0.74 after cranial tx | 6 W/cm² * after cranial tx | 3 W/cm² after cranial tx | Changes in glucose metabolism for up to more than 1 hour after sonication.

| Author et al. 2016 [17] | Human V1 | 270 | 1 ms | 500 Hz | 300 ms | 50 | 15 s (fMRI) or 2.5 s (EEG) | 2.8 * in water | 1.2 after cranial tx (maximal simulated value across N=19 subjects) | 16.6 W/cm² in water | 11.6 W/cm² after cranial tx (maximal simulated value) | 8.3 W/cm² in water * 5.8 W/cm² after cranial tx (maximal simulated value) | FMR: 11 out of 19 participants reported the perception of phosphene, and a clear fMRI response. EEG: 10/10 subjects reported phosphene sensation. Changes in VEP EEG peak.

| Author et al. 2011 [56] | Rats thalamus | 650 | 0.5 ms | 100 Hz | 20 min | 1 | - | 0.61 after cranial tx | 6 W/cm² after cranial tx | 300 mW/cm² after cranial tx | The sonication reduced the time to emergence of voluntary movement from intraperitoneal ketamine-xylazine anesthesia. A preliminary test showed that a \( I_{\text{opt}}=3.3 \text{ W/cm}^2 \) failed to decrease the duration of the anesthetic state.

<p>| Deffieux et al. 2013 [65] | Monkey frontal eye field | 320 | 1 ms | 1 kHz | 100 ms | 40 | ≥ 30 s | 1.06 in water * 0.6 after cranial tx (average across several skull) | 12 W/cm² in water* 4 W/cm² after cranial tx | 6 W/cm² in water* 13.5 mW/cm² after cranial tx | Ultrasound increased antisaccade latencies in two monkeys. |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Region</th>
<th>Parameters</th>
<th>Duration</th>
<th>Intensity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mueller et al. 2014 [66]</td>
<td>Human somatosensory cortex</td>
<td>500 ms, 0.36 ms, 1 kHz, 500 ms, 8 s</td>
<td>1.13 in water</td>
<td>23.9 W/cm² in water</td>
<td>The phase distribution of beta frequencies was altered, together with a change in phase rate of beta and gamma frequencies.</td>
</tr>
<tr>
<td>Legon et al. 2014 [61]</td>
<td>Human S1</td>
<td>500 ms, 0.36 ms, 1 kHz, 500 ms</td>
<td>7, 7</td>
<td>1.13 in water</td>
<td>23.9 W/cm² in water</td>
</tr>
<tr>
<td>Legon et al. preprint [47]</td>
<td>Human M1</td>
<td>500 ms, 0.36 ms, 1 kHz, 500 ms</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lee et al. 2018 [30]</td>
<td>Rats (anesthetized and awake) motor cortex</td>
<td>600 ms, 1 ms, 500 Hz, 300 ms, 10 s, 5-10 s</td>
<td>1.38</td>
<td>Minimum value: 2.1 W/cm², incremented by 1 W/cm², maximum value: 14.9 W/cm²</td>
<td>The amplitude of single-pulse TMS MEPs was decreased; the intracortical facilitation was attenuated; no effect on intracortical inhibition. Ultrasound reduces reaction time on a simple stimulus response task.</td>
</tr>
<tr>
<td>Yoo et al. 2017 [48]</td>
<td>Rats somatosensory cortex</td>
<td>650 ms, 0.5 ms, 100 Hz, 10 min, 1</td>
<td>-</td>
<td>4.2 W/cm²</td>
<td>Different SEP features compared to controls were evident and persisted beyond 35 min after the administration of PIU.</td>
</tr>
<tr>
<td>Yang et al. Monkey S1</td>
<td>250 ms, 0.252 ms, 2 kHz, 300 ms, 10 s, 3 s</td>
<td>1.87 in water</td>
<td>29.5 W/cm² in water</td>
<td>1.34 W/cm² in water</td>
<td>Excitation effects with BOLD</td>
</tr>
<tr>
<td>Year</td>
<td>Authors</td>
<td>Model</td>
<td>Transducer</td>
<td>f (Hz)</td>
<td>t (ms)</td>
</tr>
<tr>
<td>------</td>
<td>---------</td>
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</tr>
<tr>
<td>2018 [32]</td>
<td>Daniels et al. 2018 [39]</td>
<td>Pigs (after craniotomy) auditory and rats inferior colliculus</td>
<td>230 (1000 element transducer)</td>
<td>100 ms</td>
<td>0.333 Hz *</td>
</tr>
</tbody>
</table>
| | | | | | | | | | Rats: 0.08 * Pigs and rats: 0.17 *  
| | | | | | | | | | Rates: 2.3 W/cm² Pigs and rats: 4.6 W/cm²  
| | | | | | | | | | Rates: 765.9 mW/cm² * Pigs and rats: 1.53 W/cm² *  
| | | | | | | | | | AEP not only at the target but also off-target somatosensory and associated brain regions as a cause of modulation in downstream brain regions.  
| | | | | | | | | | Kim et al. 2018 [33] | Mice motor cortex | 183 (CMUT 32 elements, array) | 4.5 ms | 200 Hz | 200 ms | 25 | Around 9.6 s | 0.12 * before cranial tx | Up to 61.5 mW/cm² before cranial tx | Up to 55.4 mW/cm² before cranial tx | At an intensity of ISPPA=34.1 mW/cm², the average stimulation success rate of four mice was over 70%.  
| | | | | | | | | | Dallapiazza et al. 2018 [21] | Swine thalamic regions | 1.145 MHz (single element) 650 and 220 kHz (multi element phased array transducer) | 43.7 ms | 10 Hz | 40 s | 1 | - | 0.53 |  
| | | | | | | | | | Issp = 3 W/cm² with TBD=0.5 ms and PRF=100 Hz (5% duty cycle) successfully suppressed the VEP. Higher duty cycle (8.3 %) increased the VEP. The same  
| | | | | | | | | | Kim et al. 2015 [49] | Rats visual cortex | 850 | 0.5 ms | 20, 100, 166 Hz | 150 s | 1 | - | Max 0.75 |  
| | | | | | | | | | Isppa = 1 W/cm², TBD=0.5 at PRF=100Hz and Isppa=3W/cm², TBD=0.5ms, PRF=100Hz, corresponding to 50 and 30 mW/cm² Isppa did not change VEP. Isppa=3 W/cm² with TBD=0.5 ms and PRF=100 Hz (5% duty cycle) successfully suppressed the VEP.  

- **ms**: Milliseconds  
- **Hz**: Hertz  
- **W/cm²**: Watts per square centimeter  
- **AEP**: Auditory evoked potential  
- **%**: Percentage  
- **TBD**: Timing between duration  
- **PRF**: Pulsed repetition frequency  
- **Isppa**: Intensity of stimulation per pulse per area  
- **VEP**: Visual evoked potential  
- **SSEP**: Somatosensory evoked potential
<table>
<thead>
<tr>
<th>Authors</th>
<th>Location</th>
<th>Frequency</th>
<th>Pulse Width</th>
<th>Duty Cycle</th>
<th>Power Density</th>
<th>Duration</th>
<th>Study Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min et al. 2011</td>
<td>Rats thalamus</td>
<td>690</td>
<td>0.5 ms</td>
<td>100 Hz</td>
<td>180 s</td>
<td>1</td>
<td>0.33 after cranial transmission</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>2.6 W/cm² after cranial transmission</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>130 mW/cm² after cranial transmission</td>
</tr>
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<td></td>
<td></td>
<td>Suppression of the number of epileptic signal bursts. Average among all 9 rats that underwent treatment.</td>
</tr>
<tr>
<td>Baek et al. 2018</td>
<td>Mice lateral cerebellar nucleus</td>
<td>350</td>
<td>0.5 ms</td>
<td>1 kHz</td>
<td>300 ms</td>
<td>2 s</td>
<td>0.34 in water</td>
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<td>2.5 W/cm² in water</td>
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<td></td>
<td>1.25 W/cm² in water</td>
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<td></td>
<td>Enhancement of sensorimotor recovery after stroke. Decreased level of brain edema and tissue swelling in the affected hemisphere 3 days after the stroke.</td>
</tr>
<tr>
<td>Folloni et al.</td>
<td>Monkey amygdala and anterior</td>
<td>250</td>
<td>30 ms</td>
<td>10 Hz</td>
<td>40 s</td>
<td>1</td>
<td>Maximum 2.64 in amygdala and 1.64 in ACC (estimation after cranial transmission)</td>
</tr>
<tr>
<td></td>
<td>cingulate cortex (ACC)</td>
<td></td>
<td></td>
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<td></td>
<td>Maximum 51 W/cm² in amygdala and 17 W/cm² in ACC (estimation after cranial</td>
</tr>
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<td>transmission)</td>
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<td></td>
<td>Maximum 15.3 W/cm² in amygdala and 5.3 W/cm² in ACC (estimation after cranial</td>
</tr>
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<td>transmission)</td>
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<td></td>
<td></td>
<td>Enhancement of sensorimotor recovery after stroke. Decreased level of brain edema and tissue swelling in the affected hemisphere 3 days after the stroke.</td>
</tr>
<tr>
<td>Li et al. 2016</td>
<td>Mice motor cortex</td>
<td>1 MHz</td>
<td>0.5 ms</td>
<td>1 kHz</td>
<td>300 ms</td>
<td>3 s</td>
<td>260 to 460 mW/cm² after cranial transmission *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(and high</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>From 130 to 230 mW/cm² after cranial transmission *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>frequency,</td>
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<td></td>
<td></td>
<td></td>
<td>The peak EEG amplitude increased with increasing Ispta.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 MHz)</td>
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</tr>
<tr>
<td>Yang et al. 2012</td>
<td>Rats thalamus</td>
<td>650</td>
<td>0.5 ms</td>
<td>100 Hz</td>
<td>20 min</td>
<td>1</td>
<td>0.2</td>
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<td>3.5 W/cm² after cranial transmission</td>
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<td></td>
<td></td>
<td>175 W/cm² after cranial transmission</td>
</tr>
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<td></td>
<td>Extracellular GABA level started to decrease upon sonication and remained reduced compared to control group up to 100 minutes after the end of sonication. The same effect was not observed for the extracellular glutamate level.</td>
</tr>
<tr>
<td>Han et al. 2017</td>
<td>Mice motor cortex</td>
<td>850</td>
<td>0.23 ms</td>
<td>1.5 kHz</td>
<td>66.6 ms</td>
<td>2 s</td>
<td>0.1-1.16 *</td>
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<td></td>
<td></td>
<td>3.38 – 39.5 W/cm² *</td>
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<td></td>
<td></td>
<td>10 W/cm² * (for safety assessment)</td>
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<td>- all after cranial transmission -</td>
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<td></td>
<td>3.46 W/cm² (for safety assessment)</td>
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<td>- all after cranial transmission -</td>
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<td></td>
<td>The robustness of the visual observed responses increased and the latency of the response decreased with increasing Ispta. Ispta&gt;3.46 w/cm² was sufficient to induce strong motor response; no response was observed for Ispta&lt;1.6 W/cm². Ultrasound-induced motor responses were inhibited more than 20 min after ketamine.</td>
</tr>
</tbody>
</table>
injection. This was confirmed in in vitro cortical neuron sample by fluorescence calcium imaging, showing a dose-dependent effect.

Gulick et al. 2017 [45]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor cortex (after craniotomy)</td>
<td>200 ms or 3 ms</td>
</tr>
<tr>
<td>Frequency</td>
<td>1 kHz</td>
</tr>
<tr>
<td>Dose</td>
<td>Max 3.1</td>
</tr>
<tr>
<td>Effect</td>
<td>9 W/cm² or 30 W/cm² *</td>
</tr>
</tbody>
</table>

Rat motor cortex (after craniotomy)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor cortex (target to elicit motor response, not corresponding to motor cortex)</td>
<td>320 ms or 3 ms</td>
</tr>
<tr>
<td>Frequency</td>
<td>2 kHz</td>
</tr>
<tr>
<td>Dose</td>
<td>Max 3.1</td>
</tr>
<tr>
<td>Effect</td>
<td>4.5 W/cm² or 9 mW/cm²</td>
</tr>
</tbody>
</table>

US directly evokes hindlimb movement, even at short burst (3 ms) and had short latency (10 ms) and long refractory (3 s) periods. US modulation significantly suppressed forelimb and hindlimb responses following ECS for several minutes after the stimulation, but shows no short-term effect.

Younan et al. 2012 [50]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor cortex</td>
<td>320 ms or 3 ms</td>
</tr>
<tr>
<td>Frequency</td>
<td>2 kHz</td>
</tr>
<tr>
<td>Dose</td>
<td>Max 3.1</td>
</tr>
<tr>
<td>Effect</td>
<td>From 0.7 to 1.77 *</td>
</tr>
</tbody>
</table>

US isppa of 7.5 W/cm² (to have 50% response) in water. Via computer stimulation, it corresponds to 17.5 W/cm² after cranial transmission due to reverberation.

A pressure threshold of 0.79 and 0.59 MPa was required to reach 50% of responsiveness, for deep or light anesthesia stage, respectively, and the sigmoid respond was less sharp in the light anesthesia stage. These pressures corresponded to an average USppa of 7.5 W/cm².

Mehić et al. 2014 [40]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different locations in mice cortex (unfocused ultrasound or modulated focused ultrasound)</td>
<td>0.2 ms or 3 ms</td>
</tr>
<tr>
<td>Frequency</td>
<td>1.5 kHz</td>
</tr>
<tr>
<td>Dose</td>
<td>Max 3.1</td>
</tr>
<tr>
<td>Effect</td>
<td>0.45-16 W/cm² for unfocused US *</td>
</tr>
</tbody>
</table>

Increasing the Isppta increase the motor movement robustness, assessed by visual assessment with unfocused US and MFUS, and the normalized success rate in MFUS.

Table 2: Overview of the parameters used in the reviewed studies. Isppta values very often exceeded the limits for diagnostic US. Cases where the Isppta values were higher than 3 W/cm², corresponding to the limit for physiotherapeutic US, are highlighted in bold. If
needed, we calculated missing parameters from the available data stated in the paper, which we indicate by “*” in the table. When the peak pressure was reported, MI was calculated using its definition (eq. (2)), and $I_{\text{ppa}}$ in water as indicated in Figure 1 ($p=1000$ kg/m$^3$, $c=1500$ m/s). $I_{\text{pta}}$ was finally determined as $I_{\text{ppa}} \times DC$. 1) For [27], only the parameters employed in the safety tests of that study are listed here. 2) For [28], only the parameters for the main experiment are reported. 3) In [65], $I_{\text{pta}}$ was determined by using ISI instead of PRP as the total pulse duration; this strongly reduces the value. 4) For [47], MI, $I_{\text{ppa}}$ and $I_{\text{pta}}$ are not stated, but authors asserted that they used the same waveform as [61]. 5) Not clear if it is in water or after cranial transmission. 6) A spatial average of intensity of 25-30 W/cm$^2$ is used. 6) Modulated focused ultrasound means that two transducer, one driven at 2.25 MHz and the other at 1.75 MHz, producing a difference frequency at 500 kHz at the focus, and a carrier frequency of 2 MHz.
<table>
<thead>
<tr>
<th>Target</th>
<th>BBB integrity</th>
<th>Cell death, damage, brain ultrastructure and hemorrhage</th>
<th>Thermal effect</th>
<th>Behaviour</th>
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<tbody>
<tr>
<td>Lagon et al. preprint [55]</td>
<td>Human thalamus or M1</td>
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<td>Fmax</td>
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<td>Verhagen et al. [22]</td>
<td>Non-human primate SMA, PPC and pre-SMA</td>
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<td>Kim et al. 2012 [28]</td>
<td>Rat abducent nerve</td>
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<td>Sheep SM1 and V1</td>
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<td>Tufail et al. 2011 [29]</td>
<td>Rabbit (after craniotomy), SM and visual area</td>
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<td>Lee et al. 2015 [51]</td>
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<td>Lee et al. 2016 [54]</td>
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<td>Lee et al. 2016 [17]</td>
<td>Human V1</td>
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<td>Species (Condition)</td>
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<tr>
<td>Lee et al. (2018)</td>
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<td>somatosensory cortex</td>
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<td>Yang et al. (2018)</td>
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<td>Daniels et al. (2018)</td>
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<td>auditory and rats inferior colliculus</td>
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<td>Dallapiazza et al.</td>
<td>Swine thalamic regions</td>
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<td>Min et al. (2011)</td>
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<td>Baek et al. (2018)</td>
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<td>Li et al. (2016)</td>
<td>Mice</td>
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<td>Yang et al. (2012)</td>
<td>Rats</td>
<td>thalamus</td>
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<td>Tissue Description</td>
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<td>Han et al. 2017 [38]</td>
<td>Mice motor cortex</td>
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<td>Gulick et al. 2017 [45]</td>
<td>Rat motor cortex (after craniotomy)</td>
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<tr>
<td>Younan et al. 2012 [50]</td>
<td>Rat cortex (target to elicit motor response, not corresponding to motor cortex)</td>
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<tr>
<td>Mehic et al. 2014 [40]</td>
<td>Different locations in mice cortex</td>
<td>X</td>
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</table>

Table 3: Overview of the safety assessments included in the reviewed studies. The involved methods are: fluorescein isothiocyanate-dextran (FITC-Dextran), trypan blue dye (T.b.), Evans blue dye (E.B.), magnetic resonance contrast agent (MR c.a.) to assess the BBB opening; antibodies to Caspase-3, quantitative transmission electron microscopy (e.m.), hematoxylin and eosin (H&E), terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) assay, cresyl violet (c.v.), GFAP (glial fibrillary acidic protein), VAF (Vanadium acid fuchsin) and luxol fast blue dye (LFB) to monitor cell death, damage, brain ultrastructure and hemorrhage; Sensors (thermo-couple [27, 28, 48, 33, 45] or optical fiber based thermal sensor [36]), maximum temperature increase, (equation (7) to estimate $\Delta T_{\text{max}}$), magnetic resonance thermometry (MR th.) and the bioheat equation for the temperature increase; motor task (m.t.)
or other for behavioral assessments. 1) safety assessed in rats that did not undergo pentylenetetrazol (PTZ) injection to induce epileptic activity [2] or photothrombosis procedure to induce ischemic stroke [19].
Supplement to Material and Methods

Physical parameters of US waves

A sketch of an experimental setup for TFUS is shown in Figure 2A, using the stimulation of a rat as example. The ultrasound pressure wave is generated by an ultrasound transducer and delivered to the target through a guide filled with acoustic gel. At any point in space, the ultrasound pressure is a sinusoidal temporal wave (Figure 2B) with a certain center frequency ($f_c$). It is usually applied at frequencies $f_c$ between 200 kHz to 650 kHz in order to allow the soundwave to pass through the skull without being completely absorbed. At the parameter ranges interesting for human application, distortions of the sinusoidal wave shape due to non-linear effects are negligible. This can be easily seen when estimating the amount of non-linearity, as e.g. described in the International Electrotechnical Commission (IEC) standards for ultrasound measurements \[1, 2\]. A burst of waves of duration TBD (tone burst duration) is triggered, followed by an inactive period (Figure 1B). The distance between two bursts is called PRP (pulse repetition period) and a batch of consecutive bursts is a sonication (with a particular SD, sonication duration). The ratio between the TBD and the PRP defines the duty cycle (DC), usually expressed in percentage. A stimulation can consist of one or more sonications, repeated at a certain ISI (inter sonication interval). At a specific position in space, the instantaneous intensity (Figure 2C) is given by

$$I(t) = \frac{p^2(t)}{\rho c},$$  \hspace{1cm} (1)

where $p(t)$ is the acoustic pressure, $\rho$ is the mass density of the medium and $c$ is the speed of sound in the medium. The intensity can be averaged over the entire time interval ($I_{tau}$, temporal average intensity) or only over the TBD ($I_{pau}$, pulse average intensity). If these values are calculated at the position of the spatial maximum, they are called $I_{spa}$ (spatial peak temporal average intensity) and $I_{ppa}$ (spatial peak pulse average intensity).
I_{spta} can be mathematically expressed as

\[ I_{spta} = \frac{1}{PRP} \int_{0}^{PRP} I_i(t, \hat{r}_{max}) dt, \]  

(2)

where \( \hat{r}_{max} \) denotes the position of maximum intensity. For an arbitrary pulse waveform, \( I_{sppa} \) can be expressed as

\[ I_{sppa} = \frac{1}{PD} \int_{t_{10\%}}^{t_{90\%}} I_i(t', \hat{r}_{max}) dt', \]  

(3)

where \( t_{10\%} \) and \( t_{90\%} \) are the time points where 10% and 90% of \( \int_{0}^{PRP} I_i(t, \hat{r}_{max}) dt \) is reached. PD (pulse duration) is defined as \( 1.25 \times (t_{90\%} - t_{10\%}) \). In the papers considered in this review, \( I_{spta} \) is calculated as \( I_{sppa} \times DC \) during the sonication (the exception is [3], which calculated \( I_{spta} \) as \( I_{sppa} \times SD/ISI \)).

The above measures are usually determined from measurements of the TFUS pressure profile using a hydrophone in a water-tank. Skull samples are used as standard procedure to estimate the TFUS intensity in the brain after transmission through the skull bone. As this cannot account for interindividual variability, the attenuation through the temporal bone window (the thinnest area of the lateral skull) of a sample could for example be used as the worst-case scenario. This is a conservative strategy to ensure safety for all skull regions, but it will lead to low intensities in many subjects with thicker skulls. Alternatively, computer simulations have been employed, but their accuracy and precision require further testing [4, 5].

**Safety regulations for diagnostic US**

To quantify the possible harmful effect of cavitation and temperature increase in tissues, two main indices have been introduced. An estimation of the likelihood of inertial cavitation, which is a threshold phenomenon, is given by the mechanical index (MI), defined as

\[ MI = \frac{p_r}{\sqrt{f_c}}, \]  

(4)

where \( p_r \) is the peak rarefaction pressure expressed in MPa, and \( f_c \) is the center frequency expressed in MHz. MI is dimensionless, so a multiplication of a factor \( \sqrt{1 \text{MHz}/1 \text{MPa}} \) is implied (it should be noted that equation 4 is used as stated in TFUS studies, while \( p_r \) is usually derated by 0.3 dB cm\(^{-1}\) MHz\(^{-1}\) in diagnostic US to account for the difference between in-water and in-tissue acoustic attenuation). This is complemented by a thermal index (TI) to characterize the steady-state temperature increase in soft tissue during continuous sonication

\[ TI = \frac{W_p}{W_{deg}}, \]  

(5)

where \( W_p \) is the time-averaged acoustic power of the source and \( W_{deg} \) is the power needed to raise the temperature in the target tissue by 1°C, based on thermal models that are specific to the tissue type. When the
bone is close to the transducer surface, the ultrasound power is assumed to be completely absorbed by the skull and a different formulation of the thermal index (called TIC, thermal index for cranial bone) is used [6]:

\[
TIC = \frac{W_0/D_{eq}}{40 \text{ mW cm}^2}.
\] (6)

\( W_0 \) is the total output power from the ultrasound source in mW, and \( D_{eq} \) is the equivalent aperture diameter given by \( \sqrt{\frac{A_{aprt}}{\pi}} \) \( (A_{aprt} \) is the aperture area of the source) [7].

The safety guidelines for diagnostic ultrasound devices published by the FDA (Food and Drug Administration) [8] are based on MI, TI, \( I_{spta} \) and \( I_{sppa} \). Table 1 states the limits for those indices. It should be noted that there are further guidelines for other ultrasound applications, having different limits. For example, IEC standard 60601-2-5 for physiotherapy US equipment sets an upper limit for the “effective intensity”, defined as the ratio of acoustic output power to effective radiating area, of 3 W/cm². The standard also states that this value should only be reached for short times to prevent substantial heating. The “effective intensity” of 3 W/cm² is usually interpreted as upper limit for \( I_{spta} \) [9, 10, 11]. Lee and colleagues [9] compare the intensities used in their study against this limit rather than using the FDA guidelines for diagnostic US.

Estimations of temperature increases caused by TFUS

Complementary to TI, the theoretical temperature increase at the TFUS focus point has been reported. For that, the following simplified formula was used that is based on the assumption that no heat is lost by conduction, convection or other heat removal processes [12]:

\[
\Delta T_{max} = \frac{\dot{Q} \Delta t}{C_v}.
\] (7)

Variable \( \Delta t \) is the time duration of exposure and \( C_v \) is the medium’s heat capacity per unit volume \( (C_v = \rho \ C_p) \), with \( \rho \) being the mass density and \( C_p \) the heat capacity per unit mass. \( \dot{Q} \) is the rate of heat generation per unit volume and is given by

\[
\dot{Q} = 2\alpha I_{TA},
\] (8)

where \( \alpha \) is the ultrasonic amplitude absorption coefficient and \( I_{TA} \) is the temporal-average intensity. In order to find the maximum of \( \dot{Q} \), it should be calculated using the \( I_{SPTA} \). It is not clear which parameter of the TFUS should be regarded as the time duration of exposure, since two studies used TBD [13, 14], some others SD [15, 9, 16, 11], one SD x duty cycle [17], and one 1/10*SD x duty cycle [18]. A more accurate model for the temperature distribution that accounts for heat conduction and the effects of blood flow is the bio-heat equation [19], used in a recent preprint work [20].
Risk of bias

In this paragraph, we summarize possible causes of bias in the findings on TFUS safety, in terms of the treatment given to the sample, the sample itself and the method to assess safety. In most of the cases, safety assessment was performed on small animals (rodents, in particular rats), and the need to extrapolate the findings from animals to humans might bias our interpretation of the safety of stimulation settings used in humans, because of the different thickness of skull and the reverberation that might occur inside skull cavities in small animals. The length of follow-up might also be a source of bias, especially for unwanted long-term effects. In addition, within the same species, different sets of sonication parameters were applied (Table 2), and their influences on safety assessment were not confirmed by repeated experiments and damage was not assessed for all subjects. These confounds, together with possible human limitations in reading results (for example from histology) or limitation of the employed methods (e.g. spatial resolution of MRI) can give false negatives. On the other hand, false positives can occur when damages do not arise from TFUS treatment. For this reason, studies using animal models of stroke or epileptic activity [11, 21] performed histological analysis in control subjects, who underwent only TFUS treatment (Table 3). Regarding animal experiments, the environment, housing, and management are not likely to influence the results concerning anatomical changes or damage, but might affect the outcome of behavioral assessment. In human experiments, follow-up questionnaires and interviews cause a response bias that can be countered using sham stimulation in blinded trials, preferably double-blinded. Moreover, it is worth noting that 24 out of 49 papers eligible for this review didn’t assess safety or harmful effects in any way (Figure 1), which represent a lack of data.
**Title**

Identify the report as a systematic review, meta-analysis, or both.

1 – the review is a systematic review without meta-analysis of studies in both animals and humans, as indicated in the title.

**Abstract**

Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.

2- the applicable information are listed in the abstract section. ‘Data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; study appraisal and synthesis methods’ are summarized in the ‘methods’ section. ‘Limitations; conclusions and implications of key findings’ are in the ‘conclusion’ section. Given the early stage of TFUS intervention and the available data on its putative harmful effects, a systematic review registration number is not available and the authors decided not to register it.

**Introduction**

Describe the rationale for the review in the context of what is already known.

4 and 5- the rationale is described in the ‘Introduction’ section.

**Methods**

Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.

Given the early stage of TFUS intervention and the available data on its possible harmful effect, an existing systematic review registration number was not available and the authors decided not to register it.

Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.

Explained in ‘Literature review on the safety of TFUS’ (#5) and Figure 1 (#26).
<table>
<thead>
<tr>
<th>Category</th>
<th>Steps</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information sources</td>
<td>Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.</td>
<td>Explained in ‘Literature review on the safety of TFUS’ (#5) and Figure 1 (#26).</td>
</tr>
<tr>
<td>Search</td>
<td>Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.</td>
<td>Explained in ‘Literature review on the safety of TFUS’ (#5).</td>
</tr>
<tr>
<td>Study selection</td>
<td>State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).</td>
<td>Explained in ‘Literature review on the safety of TFUS’ (#5) and Figure 1 (#26).</td>
</tr>
<tr>
<td>Data collection process</td>
<td>Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.</td>
<td>Explained in ‘Literature review on the safety of TFUS’ (#5), tables 2 and 3 (#29-40).</td>
</tr>
<tr>
<td>Data items</td>
<td>List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.</td>
<td>Explained in ‘Literature review on the safety of TFUS’ (#5) and showed in tables 2 and 3 (#29-40)</td>
</tr>
<tr>
<td>Risk of bias in individual studies</td>
<td>Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.</td>
<td>Given the early stage of the investigation of TFUS safety, a systematic risk of bias assessment was not possible. However, we summarize the possible risks of bias in ‘risk of bias’ (#4 of Supplementary materials)</td>
</tr>
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<td>Summary measures</td>
<td>State the principal summary measures (e.g., risk ratio, difference in means).</td>
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<tr>
<td>Synthesis of results</td>
<td>Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I²) for each meta-analysis.</td>
<td>Not applicable: meta-analysis not performed</td>
</tr>
<tr>
<td>Risk of bias across studies</td>
<td>Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).</td>
<td>Given the early stage of the investigation of TFUS safety, a systematic risk of bias assessment was not possible. However, we summarize the possible risks of bias in ‘risk of bias’ (#4 of Supplementary materials)</td>
</tr>
<tr>
<td>Additional analyses</td>
<td>Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.</td>
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<tr>
<td>RESULTS</td>
<td>Study selection Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.</td>
<td>Described in ‘studies screened in this review’ (#12) and Figure 1 (#26).</td>
</tr>
<tr>
<td>Study characteristics</td>
<td>For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and</td>
<td>Described in ‘studies screened in this review’ (#8) and Table 2 and 3 (#29-40).</td>
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Table S1: This table shows details on our implementation of the PRISMA procedure for reporting systematic reviews [22, 23].
Bibliography


THE FALLACY OF SIMPLICITY: TRANSDUCER MODELING FOR ACCURATE ACOUSTIC SIMULATIONS OF TRANSCRANIAL FOCUSED ULTRASOUND STIMULATION

The following manuscript has been submitted to Journal of Neural Engineering.
Abstract

**Objective**: Low intensity transcranial focused ultrasound stimulation (TFUS) is emerging as non-invasive brain stimulation technique with superior spatial resolution and the ability to reach deep brain areas. Medical image-based computational modeling could be an important tool for individualized intracranial TFUS dose control and targeting optimization, but still requires further validation. This study aims to assess the impact of the transducer model on the accuracy of the simulation predictions.

**Approach**: Using hydrophone measurements, the acoustic beam of a single element focused transducer (SEFT) was characterized. The SEFT had similar specifications as those employed in prior TFUS experiments. The acoustic intensity distribution was assessed in a homogeneous water bath and after transmission through obstacles (3D-printed shapes and skull samples). The acoustic simulations employed the finite difference time domain method and were informed by computer tomography (CT) images of the obstacles. Computational transducer models of varying complexity were tested. They modeled the SEFT either as a surface boundary condition or also accounted for its internal geometry. The simulations were related to the measurements by quantitatively comparing key metrics for peak location, focus size, as well as intensity magnitude and spatial distribution.

**Main results**: While a surface boundary condition with an adapted, non-physical, ‘effective’ curvature radius could reproduce the measured focus location and size in a homogeneous water bath, it regularly failed to accurately predict the beam profile after obstacle (e.g., skull) transmission. Models that represent the internal geometry of the transducer also required a one-time calibration based on the homogeneous water bath measurement, but then performed substantially better in all cases featuring an obstacle. For one of the
tested 3D-printed obstacles, the simulated intensities deviated substantially from the measured ones, irrespective of the transducer model. We attribute this finding to a standing wave effect, and further studies should clarify its relevance for accurate simulations of skull transmission.

**Significance**: Validated transducer models are important to ensure accurate simulations of the acoustic beam of SEFTs, in particular in the presence of obstacles such as the skull.

**Keywords**:
transcranial focused ultrasound stimulation, single element focused transducer, finite difference time domain, computational dosimetry, Gamma method

### 1. Introduction

Transcranial focused ultrasound (TFUS) has been successfully applied for stereotactic neurosurgery, brain tumor ablation, and reversible blood brain barrier (BBB) disruption. More recently, TFUS has also emerged as a promising non-invasive brain stimulation technique due to the smaller focal size and the possibility to reach deeper brain areas compared to other non-invasive stimulation techniques [1]. Both excitatory and inhibitory neuropeptidary effects of TFUS have been repeatedly demonstrated in several animal species, including non-human primates [2, 3, 4, 5, 6] and humans [7, 8, 9, 10].

Safe application of TFUS requires the precise control of the ultrasound dose in the brain. To this end, most studies involving TFUS so far have employed single element focused transducers (SEFT) due to their relative ease of use and low cost, despite a more limited control of the energy deposition and spatial targeting (focus position and size) compared to multi-element phased array transducers. The SEFT’s beam profile can be characterized using hydrophone acoustic pressure measurements both in a homogeneous water environment and after the transmission through skull samples. These measurements, however, allow only for a limited assessment of the actual TFUS dose in human *in-vivo* applications as the beam profile depends strongly on the skull’s heterogeneous and individually varying structure and thickness [11]. Computer simulations informed by imaging techniques like magnetic resonance imaging (MRI) and computed tomography (CT) could play an important role in a non-invasive dose control and treatment planning on an individual patient basis. Simulations typically make use of some numerical method (e.g. the finite difference time domain - FDTD - method) to solve the pressure wave equation [12] for modeling the propagation of acoustic waves through inhomogeneous media. Their usefulness for dose control in practical applications directly depends on their accuracy.

Much prior work has focused on the geometry and acoustic properties of the skull [13, 14, 15, 16, 17, 18], as inadequate skull models will cause significant errors in transcranial simulations. However, special care also must be taken when modeling the transducer device. SEFTs typically consist of a flat vibrating piezoelectric element followed by a matching layer whose end is shaped like a concave spherical cap. However, SEFTs are usually modelled by representing only the forward-facing, spherical-cap-shaped surface and imposing a pressure or velocity boundary condition on it, neglecting the internal structure of the device. The
simulated beam profile of this idealized and simplified model differs from the real profile when modeling the transducer surface curvature according to its real curvature, as shown in Figure 3b. Manufacturers therefore frequently report an effective curvature to match the experimentally measured focus location [19] in a homogeneous water bath, as shown in Figure 3c. Simulations typically model the transducer by using this reported effective curvature or by adapting the simulated curvature to fit experimental data on the focus location, and ignore the real and internal transducer geometry [20, 11, 21, 22, 23].

While this approach allows matching the experimental location of the beam focus and its size (i.e., the peak dose location and full width at half maximum) in a test tank with water as medium and no obstacle, further validation is required when moving to more complex and heterogeneous obstacles, such as the skull. In this study, we compared the simplified effective transducer model (S_{eff}), as per the manufacturer’s specification, against physically realistic and detailed models of the transducer accounting for its internal geometry (P_{1-5}). We investigated the sensitivity of the new physical model to the different underlying parameters and assessed the accuracy of both models by comparing the results with hydrophone measurements of the beam in the presence of different obstacles. Obstacles consisted of two bone samples (sheep and pig) and three 3D-printed simple and skull-shaped phantoms made of Veroblack, a material of known acoustic properties. In addition, we acquired CT data of the obstacles with the transducer and obstacle holder for precise positioning and to derive estimates of the obstacle’s geometry and acoustic property distributions in the case of the bone skulls. Our results show that the simplified, effective transducer model produces substantial deviations between the simulations and measurements. This effect is even larger for a model that assigns a boundary condition to a surface reproducing the real, geometrically correct (physical curvature radius) transducer surface shape (S_{geom}). The deviations were substantially reduced for our new physical transducer model that was established based on its actual internal and external geometry and from calibration measurements in a pure water background.

2. Methods

2.1. Bone samples and phantoms

In this study, two animal skull samples (pig and sheep) and three phantoms were tested. Soft tissue from both skulls was mechanically removed with tweezers, and the samples were cut to maintain the upper parts of the skull. While the entire upper part of the sheep skull was preserved, only part of the pig skull was extracted to have a thickness comparable with that of the human cranium. A mostly flat surface was obtained where the pig skull was cut. The two bone samples were then glued to holders (see Figure 2) and subsequently kept under phosphate-buffered saline (PBS) solution. In addition, three 3D-printed phantoms were constructed from Veroblack (acoustic properties in Table 1). Two of those phantoms replicated the outer shape of pig and sheep skull samples, as reconstructed from CT data. The third phantom consisted of a printed rectangular slab obstacle (100 × 100 × 5 mm^3).
Figure 1: (A) The actual transducer and its physics-based model. The actual pig (B) and sheep (C) skull samples, and the respective 3D-printed phantoms (E, F). Example slice of the pig skull CT data (D) and the derived speed-of-sound map (G). Example of simulated acoustic intensity maps obtained with the pig bone (H) and sheep Veroblack (I) skull models.
Table 1: Material Properties (@ 500 kHz)

<table>
<thead>
<tr>
<th>Material</th>
<th>$\rho$ [kg/m$^3$]</th>
<th>$c$ [m/s]</th>
<th>$\alpha$ [Np/m]</th>
<th>$Z$ [$10^6$ kg/(m$^2$ s)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1000</td>
<td>1500</td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>Veroblack</td>
<td>1180</td>
<td>2495</td>
<td>21.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Acrylic Resin</td>
<td>1190</td>
<td>2750</td>
<td>7.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Acrylic M1-7</td>
<td>1180</td>
<td>2610</td>
<td>14.2</td>
<td>3.1</td>
</tr>
<tr>
<td>Human Cortical Skull</td>
<td>1908</td>
<td>2814</td>
<td>27.2</td>
<td>5.4</td>
</tr>
</tbody>
</table>

2.2. US transducer and water tank measurements

A single element spherical ultrasound transducer element (IPBD2, Hagisonic, South Korea) operating at 500 kHz, with an aperture diameter of 3 cm, a radius of curvature of 2.5 cm, and a focal length of 5 cm (incorrectly reported as radius of curvature by the manufacturer specification sheet [19]), was used to generate the acoustic pressure waves. The setup used for the bone samples employed two function generators (33220A, Agilent Technologies, California, United States) to generate a burst (20 pulses / burst) of sinusoidal waves with a center frequency of 500 kHz at a pulse repetition frequency of 1 kHz. The pulse was subsequently amplified by a power amplifier (5312, OPHIR, California, USA) and sent to the transducer. A custom-designed 3D-printed holder was used to fix the transducer inside a tank filled with de-ionized water and the pressure wave was sampled with a calibrated needle hydrophone (NH1000, Precision Acoustic, Dorset, UK) carefully inserted in a holder surrounded by a sponge in order to minimize reflection. Before each measurement series, the exact position of the transducer inside the holder was measured using a caliper. The hydrophone was moved by a stepper-motor system (Sciencetown Co., Incheon, South Korea) with a plane sampling distance of 0.25 mm and controlled by custom written software in Matlab. The signal from the hydrophone was transmitted and visualized with an oscilloscope (DSOX2022A, Agilent Technologies, California, United States). The raw data for each acquisition point were sent via USB to a computer and stored. In order to decrease noise, the signal was acquired using the average mode of the oscilloscope, with 32 samples averaged at each measurement position. For logistic reasons, the measurement setup used for the 3D-printed phantoms was changed to employ a different function generator (33500B, Keysight, California, United States), amplifier (240L, E&I, New York, United States) and oscilloscope (DSO-X 3024A, Agilent Technologies, United States), and an in-plane sampling resolution of 0.3 mm. The material of the hydrophone holder was Plexiglas in these measurements, rather than aluminum, to improve the acoustic impedance matching with water. Measurements of the beam profile in water confirmed that this change did not affect the recorded data.

Thirteen planes parallel and one perpendicular to the transducer aperture were acquired to fully characterize the beam profile. The distance between twelve of the parallel planes was 5 mm and an additional plane near the focus was acquired at a distance of 2.25 mm from the nearest plane to better sample the strong spatial variations of the beam. The aperture-perpendicular plane was chosen to traverse the beam position of maximum intensity. In the case an important secondary focus was detected, an additional perpendicular plane...
was acquired to cover it appropriately. Prior to each plane measurement, the position of maximum intensity in water was determined as follows. First, two parallel planes with a separation of 2 cm were acquired near the focus and the positions of the peak intensities in each plane determined. Subsequently, measurements were performed along a line through these two positions. The obstacle was then put in place and several planes were acquired as stated above.

2.3. Actual measurements with objects

In order to precisely position the objects in the water tank, a holder was 3D-printed for each obstacle. This holder allows for the obstacle to be screwed in place at one of 5 different locations (position 1, 2A, 2B, 2C, and 3; see Figure 2).

The exact distance between transducer and obstacle was determined as follows: First, the location of the peak was determined in a water tank. Its position was recorded and used to define the symmetry axis. Then, the obstacle and its holder were inserted in the water tank. The distance along the symmetry axis between the forward facing obstacle surface and the previously determined focus location (in the absence of the obstacle) was measured. These measurements are used to accurately determine the position of the obstacle relative to the transducer. The complete set of obstacle distances to the transducer are shown in Figure 2.

Beam profiles were measured after transmission through the two animal skull samples. A similar procedure was followed for the three 3D-printed Veroblack phantoms.

2.4. Calculation of US intensity from the measured data

The stored raw signals were first filtered with a high pass 4th order Butterworth filter with a cutoff frequency of 200 kHz to remove low-frequency noise. For each measured position, the intensity was then calculated as

\[ I = \frac{p^2}{2\rho c} \]

where \( \rho \) is the density of water (1000 kg/m\(^3\)) and \( c \) is the speed-of-sound in water (1500 m/s). The recorded data can also be used to determine the sonication phase, even though analysis of the phase was outside the scope of this study.

2.5. CT imaging of the objects

We acquired CT data of all objects attached to their holders both in water (temperature: 17.4 °C) and air backgrounds using a PET/CT scanner (positron emission tomography (PET), Biograph 128, Siemens, Germany). For each object, we acquired CT data with tube current-time product of 115 mAs and tube potential of 80 kV, which would correspond to a low dose of 0.3 mSv for a human head scan (roughly one third of the dose of clinical head scans). A sharp filter (H60s) was used during reconstruction. The nominal spatial resolution of the reconstructed images was 0.36 × 0.36 × 0.6 mm\(^3\).
Figure 2: Details of the measurement setup. (A) The orange arrow points to the transducer, which is inserted in its holder (silver plastic) to which the skull holder (gold plastic) is screwed. The obstacle (here, the sheep skull) is glued to thin acrylic rods that are fixated in holes of the main part of the holder. The white arrow points to the hydrophone. It is fixed in a holder, which is surrounded by sponge to absorb acoustic reflections. While the transducer and the skull stay in the same position throughout a measurement, the hydrophone holder is moved by a stepper-motor system. (B) Holders of the hydrophone and object seen from the side (indicated by the green arrow in A). The relative position of the skull and the transducer can be changed in steps using different screw holes. (C) A schematic of the employed configurations in this study. Black dots indicate the position of the screws. The horizontal distance of the holes in the skull holder is 1 cm, while the vertical one is 8 mm.
2.6. Simulation framework

Acoustic propagation was simulated within the Sim4Life (ZMT Zurich MedTech AG, Zurich, Switzerland) platform for computational life sciences, which encompasses functionality for image-based modeling, a range of acoustic propagation solvers, a Python scripting interface, as well as post-processing, visualization, and analysis functionality. For the purpose of this study, the linear acoustic pressure wave solver (LAPWE) from [12] was employed, which implements FDTD on rectilinear, inhomogeneous meshes and supports multiple graphical processing units (GPU) parallel execution to permit simulation of models with a large number of degrees-of-freedom within reasonable time. The LAPWE solver solves the wave equation:

\[ \rho \nabla \frac{1}{\rho} \nabla p - \frac{1}{c^2} \frac{\partial^2 p}{\partial t^2} - \tilde{\alpha} \frac{\partial p}{\partial t} = 0 \]

\[ \tilde{\alpha} = 2\alpha \sqrt{\frac{\alpha^2 c^4}{\omega^2} + c^2} \]

where \( \rho \) is density, \( c \) is speed-of-sound, \( \alpha \) is attenuation, \( p \) is pressure, \( t \) is time, and \( \omega \) is angular frequency. This equation accounts for reflections due to acoustic impedance (\( Z = \rho c \)) variations and discontinuities, standing waves, and the combined impact of absorption and scattering. However, it neglects shear waves in the rigid skulls, as well as tissue non-linearities, which can lead to frequency mixing and higher harmonics, but are not relevant at the studied intensities [13]. Grid generation ensured that the voxel size in every material remained below a tenth of its wavelength. The coarsest grid step outside the skull region was 0.3 mm, while it was 0.1 mm in the skull region, resulting in a simulation mesh with about 500 million voxels. To ascertain the suitability of the discretization, a grid convergence analysis was performed, where the grid resolution was increased until the peak amplitude change remained below 1%. Perfectly Matched Layers (PML) boundary conditions with 16 layers were used to ascertain that reflections at the domain boundary remain negligible (< 2% amplitude change [24]). Detailed information about the verification and validation of this solver and its implementation can be found in [24].

Simulations of the experimental setup were performed for a pure water background, as well as for the two skulls and the 3D-printed Veroblock plate and skulls, at up to five positions each (see Figure 2). The skull models were generated by thresholding the normalized CT images at 500 Hounsfield Units (HU) and extracting the skull component. Image up-sampling (from 0.36 mm base resolution to 0.111 mm resolution) and surface smoothing (sigma = 1 mm) were applied to avoid surface staircasing related to the CT resolution which is large compared to the discretization step in the simulation domain (0.1 mm). The transducer holder geometry was readily available from the computer-aided-design model used for rapid prototyping. For more details on the transducer geometry modeling, see Section 2.7. Reflections from the hydrophone and the tank walls were not simulated and had a very low impact on the measurement data because of the use of pulsed rather than continuous sonication. Using sufficiently short pulses prevents returning (reflected) waves from interfering with the outgoing wave in the measurement volume, due to the differing arrival times.
Water and air were assigned acoustic properties according to the IT’IS database [25] and Veroblack according to [11]. For the acrylic transducer matching material, properties were assigned within the range provided for acrylic resin and acrylic M1-7 in [26]. Transducer matching layer properties are discussed in Section 2.7. The employed values can be found in Table 3 and the matching layer variations in Figures 5 and 6. To simulate the inhomogeneity of the bone skulls, the approach from [16] was used, which assumes that the CT HU can be linearly mapped into bone density, which in turn maps to speed-of-sound according to the following linear relations:

\[
\rho = \rho_{\text{ref}_1} + \frac{\rho_{\text{ref}_2} - \rho_{\text{ref}_1}}{H_{\text{ref}_2} - H_{\text{ref}_1}} HU
\]

\[
c = c_{\text{ref}_1} + \frac{c_{\text{ref}_2} - c_{\text{ref}_1}}{\rho_{\text{ref}_2} - \rho_{\text{ref}_1}} \rho
\]

where \(\text{ref}_1\) and \(\text{ref}_2\) refer to the reference values used to anchor the linear mappings. \(\text{ref}_1\) was chosen as water, and \(\text{ref}_2\) was set to species specific average skull properties (density and speed-of-sound). For simplicity, attenuation in the skull was set to a constant value that was experimentally adjusted to match the maximum measured intensity when the skull was placed in the holder’s first position. Typically, acoustic skull attenuation is experimentally inflated and adjusted to account for microscopic backscattering effects that cannot be effectively captured by even high resolution CT images [11, 16, 27]. As discussed in Section 4, attenuation primarily impacts the overall field scaling, but has little impact on focus shape and position. Skull mapping parameters are provided in Table 2. A histogram of the skull CT HU data was extracted and clipped at the HU of water (0 HU). The very apparent subsequent peak towards higher HU was assumed to be the average HU in skull and assigned to the corresponding (species-specific) value, as found in the literature ([28] for pig, [22] for sheep).

HU-based property assignment was restricted to the skull region and linear interpolation was used to relate the finer voxel resolution (0.1 mm) to the coarser CT (0.335 mm). To efficiently handle inhomogeneity, voxels were assigned to 20 different bone classes based on HU binning. This permits precomputation and storage of update coefficient look-up tables for the GPU accelerated solver, thus increasing solver efficiency. An increase of the number of binning classes beyond 20 was found to not significantly affect the acoustic distributions anymore (< 1% change in peak intensity). The impact of geometry segmentation and inhomogeneous mappings using different CT scanner parameters is analyzed in [29].

2.7. Transducer modeling

Acoustic sources were modeled as time-harmonic Dirichlet pressure boundary conditions. The transducer casing was treated as perfect reflector (zero pressure).

Initially, the transducer was modeled as a spherical cap, with an aperture diameter of 30 mm and an outer surface curvature radius of 50 mm as provided in the manufacturer specification sheet (\(S_{\text{eff}}\)). However, this curvature was found to not correspond to the real transducer surface shape (radius of curvature of 25 mm), but rather to an effective distance to
the focal spot (50 mm). Thus, the shape of the transducer cross-section profile was measured and used as pressure boundary condition ($S_{\text{geom}}$). This was also found to not reproduce the measured pressure distribution in water (see Section 3.1 and Figure 3), and subsequent inquiry with the manufacturer revealed that the actual piezo element is in fact a flat disk and that an additional acrylic element with the measured surface shape is inserted on top of this disk (Figure 3). Hence, a detailed transducer model was constructed that features a flat disk, which is assigned a time-harmonic pressure distribution, while the curved acrylic resin element on top (matching layer) is treated as a passive medium that shapes the wavefront (Figure 1). The acoustic properties (density, speed-of-sound, and attenuation) of the acrylic matching layer were based on [26] and varied within the associated uncertainty range until simulation results matched the measured field in the obstacle-less water bath setup. The depth of the disk was set to a quarter of the wavelength in the matching material as reported in [30] and also varied. Furthermore, a radial dependence of the pressure profile on the disk was introduced (linear, cosine, and spherical) to reflect the potential impact of the transducer walls on the vibrational mode (‘aperture function’) of the piezo source.

The parameters were varied within the uncertainty range of acrylic material properties (see Figures 5 and 6) to get five candidate physical transducer models ($P_{1-5}$) that match the measured field in the obstacle-less water bath, using the Gamma metric introduced below as criterion (see Table 3). These fitted ‘physical’ models and the ‘effective’ model were then used to analyze how well they are able to predict transcranial ultrasound intensity distributions.

<table>
<thead>
<tr>
<th>Model</th>
<th>$c$ [m/s]</th>
<th>$\alpha$ [Np/m]</th>
<th>Aperture</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_1$</td>
<td>2660</td>
<td>14.28</td>
<td>Spherical</td>
</tr>
<tr>
<td>$P_2$</td>
<td>2600</td>
<td>14.28</td>
<td>Cosine</td>
</tr>
<tr>
<td>$P_3$</td>
<td>2600</td>
<td>28</td>
<td>Spherical</td>
</tr>
<tr>
<td>$P_4$</td>
<td>2660</td>
<td>50</td>
<td>Spherical</td>
</tr>
<tr>
<td>$P_5$</td>
<td>2700</td>
<td>40</td>
<td>Spherical</td>
</tr>
</tbody>
</table>

Table 3: Transducer parameters of the best five physical candidate models. Parameters $c$ and $\alpha$ are the speed of sound and attenuation properties of the acrylic matching layer. ‘Aperture’ corresponds to the radial pressure profile applied to the piezo element disk. For all of these models, the density of the acrylic matching layer was set to $\rho = 1180$ kg/m$^3$ and the distance of the piezo element (in wavelengths $\lambda$) from the nearest transducer surface point was set to $d = 0.25\lambda$. 

<table>
<thead>
<tr>
<th>Skull</th>
<th>$ref_1$ (Water)</th>
<th>$ref_2$ (Average Skull)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HU</td>
<td>$\rho$ [kg/m$^3$]</td>
<td>$c$ [m/s]</td>
</tr>
<tr>
<td>Pig</td>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>Sheep</td>
<td>0</td>
<td>1000</td>
</tr>
</tbody>
</table>

Table 2: Parameters used for skull property mappings, based on the approach from [16], but using species specific values from [28] and [22].
2.8. Metrics

All simulated pressure distributions were normalized by the peak simulated pressure in the absence of a bone or printed skull obstacle. The following metrics were used for quantification and comparison purposes: (i) $d_z$: peak location as a measure of focus position; (ii) $I_{peak}$: peak intensity (normalized by the peak intensity in the absence of any obstacle); (iii) $\text{FWHM}_z$: extent of the focus full width at half maximum along the principal propagation axis (where available); (iv) $\text{HWHM}_{z+}$: half maximum past the location of the focus (or the length until the intensity has decayed by a factor of two, in case the obstacle prevents measuring the peak location); (v) Gamma: Gamma comparison value.

The Gamma comparison method was proposed by [31] to compare planned and administered radiological dose distributions. It permits to quantitatively compare fields that include both shape distortions and variations in amplitude, and its use in validation experiments has been advocated by [24]. Given spatial tolerances and an amplitude tolerance ($\Delta d_{x,y,z}$ and $\Delta D$, respectively), the gamma index ($\gamma$) compares every measurement point with all simulation points and finds the simulation point that minimizes an Euclidean distance norm combining distance and value ($f(\vec{r})$) deviations (normalized by the corresponding tolerances):

$$\Gamma (\vec{r}_{\text{model}}, \vec{r}_{\text{meas},i}) = \sqrt{\frac{(f_{\text{model}}(\vec{r}_{\text{model}}) - f_{\text{meas}}(\vec{r}_{\text{meas},i}))^2}{\Delta D^2} + \sum_{j=x,y,z} \frac{(r_{j,\text{model}} - r_{j,\text{meas},i})^2}{\Delta d_j^2}}$$

The minimized Euclidean distance norm is subsequently assigned as the score of the corresponding measurement location:

$$\gamma (\vec{r}_{\text{meas},i}) = \min \{\Gamma (\vec{r}_{\text{model}}, \vec{r}_{\text{meas},i})\} \forall \vec{r}_{\text{model}}$$

A value of 0 corresponds to a perfect match for this point and a value of 1 reflects the limit of what lies within the total tolerance. The total tolerance is obtained as root-sum-square reflecting the simplified assumption of statistical independence. We report the gamma comparison value ‘Gamma’ as the percentage of measurement points that have been assigned a norm exceeding 1 (outside of the tolerance). Furthermore, the spatial distribution of $\gamma(\vec{r}_{\text{meas}})$ provides an intuitive visualization of disagreement locations (see Figure 4 for an illustrative example of the Gamma comparison method).

$$\gamma (\vec{r}_{\text{meas},i}) > 1 : \text{disagreement exceeds combined tolerance}$$

The chosen agreement criteria were motivated by the intended application – i.e., the spatially precise targeting of a small cortical patch – and the physical beam properties. Given a focus size with a FWHM of 42 mm along the beam axis and 5 mm perpendicular to it (determined for pure water, see Figure 3), we set $\Delta d_z = 5$ mm and $\Delta d_{xy} = 2$ mm as upper thresholds for shifts of the focus position in these directions. Shifts exceeding these criteria would result in the undesired stimulation of a neighboring cortical patch, or in a peak position that is in cerebral spinal fluid (CSF) or white matter, rather than in gray matter.
Figure 3: Transducer Models: (Top) Normalized acoustic intensity along the symmetry axis for different transducer models, compared to measurement data. The Full Width at Half Maximum extent of the measured focus is indicated by vertical green line. (A-I) Measured and simulated normalized intensity distributions. (A) Measurement, (B) $S_{\text{geom}}$, (C) $S_{\text{eff}}$, (D-I) $P_{1-5}$ physical transducer models.
Based on the approximately sigmoidal dependence of TFUS intensity on neural response as demonstrated in [32], a 15% difference in peak intensity maximally changes the stimulation success rate by 10%. For this reason, $\Delta D = 15\%$ was set as the amplitude tolerance.

![Image of a figure with panels A, B, C, and D, each showing different aspects of measurement and comparison.]

**Figure 4:** Example of Gamma comparison. (A) Measurement; (B) $S_{\text{eff}}$, $S_{\text{geom}}$; (C) Gamma comparison distribution ($\gamma(r)$; tolerances: 5 mm longitudinal, 2 mm transverse, and 15% intensity); (D) FWHM of measurement profile in red, iso-curves of $\gamma(r)$ at 100% in purple and at 50% in blue. Notice that the biggest differences for $S_{\text{eff}}$ occur in the near field and in the region of the focus – the latter indicating that the measurement focus does not align perfectly with the symmetry axis. A $\gamma$ value below 100% indicates deviation within the combined tolerance. The purple iso-curve demarks the regions that exceed the combined tolerance. For $S_{\text{geom}}$, almost the entire region of the measurement focus is outside the acceptable tolerance.

### 3. Results

#### 3.1. Effects of transducer modeling on the acoustic beam in a pure water background:

Modeling the transducer as a pressure Dirichlet boundary condition following the real shape of the curved transducer surface ($S_{\text{geom}}$) results in large focus shifting, focus size mismatches, and an incorrect prediction of the spatial distribution (tolerance normalized quantities: Gamma = 20%, $dz = -348\%$, FWHM$_z = -441\%$; see Table 4). Using an ‘effective’ model that adapts the curvature radius in accordance with the effective radius provided by the manufacturer ($S_{\text{eff}}$) mostly corrects the focus shift, results in a low Gamma error (0.4%), and improves the prediction of the focus size. The physics-based transducer models further improve the agreement (Gamma = 0%), yet this is expected as they have been tuned to match water measurements. The impact of the various transducer parameters on the intensity distribution and along the symmetry axis is depicted in Figures 5 and 6.
Figure 5: Variation of the normalized intensity distribution of a physical transducer model resulting from changes: (B-E) to the aperture function (radial pressure variation of the piezo element boundary condition; constant, linear, cosine, and spherical, respectively), and (F-H) to the speed-of-sound of the acrylic matching layer (2600, 2660, and 2750 m/s). (A) The measurement reference. (Top) Plot along the symmetry axis.
Figure 6: Variation of the normalized intensity distribution of a physical transducer model resulting from changes: (B-D) to the acrylic matching layer attenuation (14, 28, and 40 Np/m), and (E-G) to the matching layer thickness (0.125, 0.25, and 0.5 wavelengths). (A) The measurement reference. (Top) Plot along the symmetry axis.
Figure 7: Sample measurement and simulation intensity distributions, and gamma comparison distribution past obstacles. (A-D) Illustrative ‘good’ simulation result (sheep skull, position 1) and (E-H) ‘bad’ simulation match (sheep skull, position 3) with measurement. (A,E) Measurement, (B,F) $S_{\text{eff}}$, (C,G) $P_2$ physics-based transducer model, (D,H) Gamma comparison of measurement and $S_{\text{eff}}$ (top) and $P_2$ physics-based transducer model (bottom). The overlaid red contour denotes the Half Maximum iso-contour of the measurement distribution; the purple contour indicates the region with a deviation that exceeds the combined tolerance.
Table 4: Comparison between the measured and simulated intensity in a watertank setup without obstacle. The differences are expressed in percentages normalized to the tolerances from Section 2.8.

<table>
<thead>
<tr>
<th></th>
<th>Gamma</th>
<th>d_z</th>
<th>FWHM_{z}</th>
<th>HWHM_{z,+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>S_{geom}</td>
<td>19.9</td>
<td>-347.7</td>
<td>-441.3</td>
<td>-399.13</td>
</tr>
<tr>
<td>S_{eff}</td>
<td>0.4</td>
<td>-87.9</td>
<td>-156.8</td>
<td>-81.48</td>
</tr>
<tr>
<td>P_1</td>
<td>0.0</td>
<td>-31.8</td>
<td>-70.9</td>
<td>0.7</td>
</tr>
<tr>
<td>P_2</td>
<td>0.0</td>
<td>-28.8</td>
<td>37.1</td>
<td>33.7</td>
</tr>
<tr>
<td>P_3</td>
<td>0.0</td>
<td>-31.8</td>
<td>-51.8</td>
<td>27.7</td>
</tr>
<tr>
<td>P_4</td>
<td>0.0</td>
<td>-31.8</td>
<td>-68.8</td>
<td>6.7</td>
</tr>
<tr>
<td>P_5</td>
<td>0.0</td>
<td>-31.8</td>
<td>-104.8</td>
<td>-47.2</td>
</tr>
</tbody>
</table>

3.2. Transmission through the Veroblack plate

Once the Veroblack plate obstacle is introduced, the ‘effective’ transducer model fails to reproduce focus location and size. Four of the five physics-based transducer models, however, are capable of predicting the focus location, size, and overall pressure distribution reliably (0% disagreement of the Gamma metric vs. 15% of the points failing the Gamma comparison for the effective transducer model; see Table 5).

3.3. Transmission through the Veroblack 3D-printed skulls

Pig (thick skull). The 3D-printed pig skull is the thickest Veroblack obstacle (the thickness along the propagation axis can exceed 10 mm), resulting in standing wave patterns inside the obstacle. While one side is mostly flat (due to the cutting), the other is slanted such that small shifts in predicted focus position can result in clear changes of the standing waves in the obstacle and the related transmission efficacy (see Section 4). Depending on the skull shift perpendicular to the propagation direction (positions 2A-C), the beam goes through the maximal thickness or partly passes outside the skull fragment border (2C). This results in two separate, prominent focal lobes and some weak secondary foci. In all the Veroblack printed pig skull cases, the peak intensity is within the (unmeasurable) region inside the skull or near its surface. Therefore, while the Gamma comparison is meaningful, reported peak intensity (I_{peak}) and focus size comparisons (HWHM_{z,+}) are not. The ‘effective’ model is unable to predict focus intensity (the error is smaller than the tolerance only for position 3) and frequently fails to correctly predict focus extent. Consequently, it displays poor Gamma metrics. The physics-based transducer models produce good results, except for positions 2B and 3, where the intensity is off, despite very good agreement in the relative distribution pattern. The physics-based simulations are all well able to handle the challenging 2C case, where the focus is right at the border of the skull fragment (see Table 6).

Sheep. The 3D-printed sheep skull is thinner and curved and the agreement between simulations and hydrophone intensity measurements is better than for the 3D-printed pig skull. The physics-based models outperform the ‘effective’ model, based on the Gamma criterion,
and with the exception of position 1, where the ‘effective’ model shows a > 30% Gamma error rate, the Gamma criterion remains below 5% for all transducer models. However, the focus position error frequently exceeds the chosen 5 mm tolerance (see Table 6).

3.4. Transmission through the bone skull samples

Pig. The physics-based transducer models show near perfect Gamma metrics (always < 1%), whereas the percentage of data-points failing to agree with the hydrophone measurements (according to the Gamma criterion) can reach over 20% for the ‘effective’ transducer model. While the physics-based transducer model occasionally slightly exceeds the prescribed peak intensity tolerance of 15%, the ‘effective’ model nearly always fails and exceeds the tolerance threshold by up to 4.8 times (see Table 7).

Sheep. Simulation performance with the sheep skull obstacle is inferior to the accuracy achieved for the pig skull. However, the physics-based models again clearly outperform the ‘effective’ model. The latter fails to pass the Gamma criterion for 7-40% of the measurement points, while the failure rate of the former is in the 4-20% range (see Figure 7). In most cases, the prediction of the peak intensity is insufficient (considering the defined 15% threshold), which seems to be related to the simulations predicting two distinct but overlapping intensity peaks, while the measurements show a single, merged peak of combined (higher) intensity (see Table 7).

4. Discussion

Translatability of transducer model. While it is correct that the physical transducer model has been optimized to fit measurements, it is important to note that this optimization was only performed for the water measurements and the fitted model was subsequently used without further adaptation for all the different obstacle setups (bone and Veroblock obstacles).

‘Effective’ and physical transducer model. The results demonstrate that using the measured transducer surface geometry as pressure source fails to correctly predict the intensity distribution and results in strong deviations in focus location and shape. Applying the ‘effective’ model geometry provided by the manufacturer or artificially varying the curvature, as frequently done [20, 11, 21, 22, 23], permits to mimic focus location, but results in important deviations in focus shape, both in terms of primary focus size and secondary foci in the near-field. Furthermore, the fact that this model is an unphysical model means that it is an ‘effective’ model only in the absence of obstacles. As the overall intensity distribution provided by the ‘effective’ model deviates substantially from that of the real transducer, the presence of the obstacle results in a completely different interference pattern, that prevents reliable modeling even of focus location (see Figure 4). In contrast, the simulations based on the physical transducer model were substantially more accurate for all tested cases after the model was calibrated to fit the measurements obtained for the pure water background. As such, similarly to the ‘effective’ model, our proposed approach for setting up the physical transducer model requires initial reference measurements, but subsequently results in more
accurate predictions of the acoustic beam. Accurate transducer modeling is also expected to
be a prerequisite for simulation-driven design of acoustic lenses placed on top of transducers,
as in [33, 34].

Transducer modeling in water. Different parameters of the physical transducer model had
specific, distinguishable impacts on the intensity distribution (see Figures 5 and 6). Changing
the attenuation parameters (of the matching material, but also of the obstacle) hardly
affected the intensity distribution and only resulted in a change in magnitude scale. Both
modification of the matching material speed-of-sound and of the piezo element depth have
a similar effect, namely a noticeable impact on the intensity distribution. This is due to the
fact that a change in speed-of-sound in the region where the wave is still mostly traveling
paraxially corresponds to an effective change in the traveled distance. The curved surface
primarily produces a radial distance dependent phase delay, which is slightly affected by
the speed-of-sound. The mechanical construction of the transducer (e.g., transducer walls
and housing) affect the vibrational modes. We have considered the impact of such an effect
on the acoustic pressure wave by introducing an aperture function. A change in aperture
function has a small impact on focus sharpness, but its main effect is to modify the location
and occurrence of secondary foci.

We have modeled the piezo element as time-harmonic Dirichlet boundary condition (pre-
scribed pressure). This is the natural and common choice when using FDTD. However, other
methods exist where boundary conditions are commonly defined in terms of prescribed ve-
locities. Constant velocity and constant pressure are not equivalent, as evident, e.g., when
looking at the analytical solution for a vibrating circular disk obtained using Rayleigh-
Sommerfeld integrals [12] where velocity is constant across the transducer surface, while
pressure is not. This distinction is typically ignored in the applied modeling literature.
However, it should be investigated further as the different boundary conditions will give rise
to different intensity distributions.

Sensitivity and tolerances. In line with [24], the sensitivity analysis presented in the results
section demonstrates that relatively small variations of parameters, such as speed-of-sound,
can have an important impact on the complex interference pattern of (curved) acoustic
transducers. Therefore, it is important to reduce uncertainty by properly characterizing the
acoustic properties of relevant materials in the sonication setup.

The choice of the tolerances (\(\Delta d_{x,y,z}\) and \(\Delta D\)) for the Gamma metric – which is used
in this study to judge simulation-measurement agreement – is driven by application specific
criteria. That is, prediction errors above the tolerances would significantly compromise the
accuracy and precision of the conclusions with regards to the targeted position in the brain
and the intensity at the target. This approach is unlike that followed in [24] where agreement
tolerances were based on a thorough uncertainty analysis. The Gamma tolerances obtained
in [24] at similar frequencies (550 kHz rather than 500 kHz) through uncertainty analysis
of sonication in the absence of an obstacle (1.3 times the wavelength in water, i.e. 4 mm;
14% of the peak intensity) are comparable to the ones used in this study (5 mm, 15%).
However, a similar uncertainty assessment in the presence of skull obstacles would have
resulted in much larger tolerances. Therefore, the approach chosen for this paper results in much stricter criteria that are hard to meet but reflect application needs.

**Standing waves.** The large difference in acoustic impedance between Veroblack and water (see Table 1) give rise to strongly reflecting interfaces (32% of the pressure amplitude for a plane wave with normal incidence). This leads to a standing wave effect within the obstacle, which results in resonator behavior (similar to that known from Fabry-Pérot resonators in laser-physics [35]) and is known to be associated with fluctuations in transmitted power as a function of the effective cavity length (see Figure 8). When the planar Veroblack slab is placed at the focus location, varying the frequency (or equivalently the wavelength or obstacle thickness) results in up to 20% changes in pressure transmission (> 40% change in intensity, see Figure 8). This standing-wave effect helps explain the focus intensity differences observed with Veroblack obstacles (see Section 3.3). Further research should be performed to assess the relevance of such findings for transcranial sonication. It is expected that skull heterogeneity and losses related to absorption or scattering reduce the occurrence of standing waves. On the other hand, the higher density and speed-of-sound of cortical bone compared to Veroblack – and the related increase in acoustic impedance mismatch – leads to a more than fourfold variability of the transmitted intensity for relatively moderate changes in speed-of-sound assignment and skull thickness modeling (computed analytically for a homogeneous cortical bone plate of realistic thickness immersed in water; see Figure 8). This results in a high sensitivity to modeling errors.

**Limitations and further work.** A ‘first principles’ approach would consist in performing proper mechanical modeling (i.e., full dynamic stress-strain simulation) of the transducer, from which the vibrational mode would emerge and might allow for accurate predictions of the acoustic beam without the need for reference measurements. However, this is beyond the scope of this paper.

The simulated sonication intensities for the bone skull samples agreed satisfactorily with the measurements only after resorting to species-specific skull attenuation maps (see Table 2). This might reflect different bone compositions between species, which can result in different properties for similar HU values. A more detailed evaluation of CT image-based modeling of skull properties is the subject of a companion paper [29].

5. Conclusions

Careful transducer modeling and experimental validation is crucial for the reliable simulation of TFUS fields, and the currently commonly employed approaches – i.e., assigning a boundary condition to the real shape of the transducer surface, or using an ‘effective’ transducer shape model that has been constructed to produce a focus at the right location in a homogeneous water setup – are inadequate for extended, curved, or complex transducers. This is particularly true in the presence of acoustic obstacles and inhomogeneity. Even physics-based transducer modeling can sometimes fail to reach the chosen, clinically motivated, agreement criteria (15% peak intensity, 5 mm for focus position and length). An optimal, but highly demanding and typically impracticable approach would include complete
Figure 8: Impact of the standing wave effect on transmission. (Top) Analytically computed plane wave (pressure) transmission factor past a flat, homogeneous obstacle (blue: Veroblock, red: cortical bone), as a function of obstacle thickness and for different attenuation factors. (Bottom) Pressure distribution obtained for a physical transducer model past an obstacle of varying thickness (5–7.5mm, corresponding to 1–1.5 wavelengths in the obstacle). The color-bar remains identical. Notice that the top figure is computed for plane-wave exposure, while the bottom figure shows results for a focused sonication.
mechanical modeling of the transducer with its housing and fixation. However, a compromise combining improved acoustic modeling of the transducer and its internal structure with an aperture function to account for the missing mechanical modes can be an acceptable solution, but requires the acquisition of reference data using hydrophone measurements. If possible, experimental effort should be invested in characterizing sensitive material properties of the transducer components and obstacle media (particularly speed-of-sound and species-specific attenuation). Comprehensive uncertainty assessment should typically be performed along with computational modeling. Standing wave effects have been found to have a high impact on the sensitivity and accuracy of transmitted intensity predictions for the 3D-printed and other homogeneous obstacles (up to fourfold variation in cortical skull material, for relatively small speed-of-sound assignment errors). Additional studies should be performed to investigate how much skull heterogeneity affects the formation of standing-waves.

<table>
<thead>
<tr>
<th>Gamma</th>
<th>I_\text{(peak)}</th>
<th>(dz)</th>
<th>FWHM(_z)</th>
<th>HWHM(_{z+})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VB Plate 2cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S(_{eff})</td>
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<td>-77.1</td>
<td>-134.9</td>
<td>-125.2</td>
</tr>
<tr>
<td>P(_1)</td>
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<td>-22.4</td>
<td>-71.9</td>
<td>-84.9</td>
</tr>
<tr>
<td>P(_2)</td>
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<td>5.4</td>
<td>-71.9</td>
<td>-6.8</td>
</tr>
<tr>
<td>P(_3)</td>
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<td>-39.6</td>
<td>-41.9</td>
<td>-67.9</td>
</tr>
<tr>
<td><strong>VB Plate 3cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S(_{eff})</td>
<td>14.9</td>
<td>-37.4</td>
<td>-11.5</td>
<td>-</td>
</tr>
<tr>
<td>P(_1)</td>
<td>0.0</td>
<td>-67.6</td>
<td>-2.6</td>
<td>-</td>
</tr>
<tr>
<td>P(_2)</td>
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<td>-29.6</td>
<td>-</td>
</tr>
<tr>
<td>P(_3)</td>
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<td>-89.8</td>
<td>-29.6</td>
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</table>

Table 5: Comparison of the simulated and measured intensity distributions with the Veroblack plate obstacles (the tolerance-normalized deviation in %).
<table>
<thead>
<tr>
<th></th>
<th>$S_{\text{eff}}$</th>
<th>$\Gamma_{\text{peak}}$</th>
<th>$d_\text{z}$</th>
<th>FWHM$_\text{z}$</th>
<th>HWHM$_{\text{z}+}$</th>
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</thead>
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<td></td>
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<td></td>
<td></td>
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<td>$P_1$</td>
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<td>-42.2</td>
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<td>-65.1</td>
</tr>
<tr>
<td>$P_2$</td>
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<td>-</td>
<td>-60.9</td>
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<td>$P_3$</td>
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<td>100.5</td>
<td>-41.2</td>
<td>-</td>
<td>-57.0</td>
</tr>
<tr>
<td><strong>VB Pig 2A</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>$P_1$</td>
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<td>-530.0</td>
<td>-32.2</td>
<td>-</td>
<td>-161.5</td>
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<td>$P_1$</td>
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<td>-</td>
<td>-72.3</td>
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<td></td>
<td></td>
</tr>
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<td>-127.2</td>
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<td>-77.4</td>
<td>-</td>
<td>-103.4</td>
</tr>
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<td>$P_3$</td>
<td>8.0</td>
<td>-113.1</td>
<td>-77.4</td>
<td>-</td>
<td>-83.9</td>
</tr>
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<td><strong>VB Pig 3</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>-102.5</td>
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<td>-38.2</td>
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<td>-35.2</td>
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<td>27.3</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$P_1$</td>
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<td>-122.2</td>
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<td>-11.3</td>
</tr>
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<td><strong>VB Sheep 3</strong></td>
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<td>-136.1</td>
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</tr>
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<td></td>
<td>0.7</td>
<td>80.1</td>
<td>-136.1</td>
<td>-</td>
<td>48.3</td>
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</table>

Table 6: Comparison of the simulated and measured intensity distributions with the Veroblack printed skull obstacles (the tolerance-normalized deviation in %).
<table>
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<tr>
<th></th>
<th>Gamma</th>
<th>I_{peak}</th>
<th>dz</th>
<th>HWHM_{z+}</th>
</tr>
</thead>
<tbody>
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<td>Pig 1</td>
<td>S_eff</td>
<td>16.4</td>
<td>-134.7</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>P_2</td>
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<td>10.6</td>
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<td>2.5</td>
</tr>
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<td>S_eff</td>
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<td>P_2</td>
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<td>-11.4</td>
</tr>
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<td>-143.7</td>
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<td>-11.4</td>
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<tr>
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<td>P_2</td>
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<td>Pig 2C</td>
<td>S_eff</td>
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<td>P_2</td>
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<td></td>
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<td></td>
<td>P_3</td>
<td>0.7</td>
<td>-113.6</td>
<td>-4.4</td>
</tr>
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<td>Sheep 1</td>
<td>S_eff</td>
<td>38.7</td>
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<td>-91.0</td>
</tr>
<tr>
<td></td>
<td>P_2</td>
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<td>25.2</td>
<td>-27.5</td>
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<td></td>
<td>P_3</td>
<td>11.8</td>
<td>78.1</td>
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<td>106.5</td>
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<td>19.1</td>
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<td>1.7</td>
</tr>
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</table>

Table 7: Comparison of the simulated and measured intensity distributions with the real skull obstacles (the tolerance-normalized deviation in %)
References

[18] Christopher W. Connor, Greg T. Clement, and Kullervo Hynynen. A unified model for the speed...


APPENDIX

C

PHYSICS BASED, VALIDATED RELIABLE MODELING
OF SINGLE ELEMENT FOCUSED ULTRASOUND
TRANSDUCER (SEFT)

The following abstract was accepted for the 19th International Symposium of ISTU
- 5th European Symposium of EUFUS.
OBJECTIVES

Transducer models for the simulation of transcranial focused ultrasound stimulation (TFUS) are often not accurate when only based on the specifications of the manufacturer, but require adaptations based on hydrophone measurements. We investigated the importance of creating a transducer model that is based on a real physical representation of the geometry and internal transducer structure, rather than an ‘effective’ model optimized to fit hydrophone measurements in water.

METHODS

A SEFT operating at 500 KHz has been characterized through measurements in a water tank with and without obstacles of varying shape (plate, pig and sheep skull) printed from a material with known acoustic properties (Veroblack) at different positions. We compared an ‘effective’ model with our new physical model accounting for internal structure, using the gamma method (spatial and intensity tolerance: 5mm and 15%). We calculated the percentage of points outside this tolerance (failure %) as well as the deviations of the position of maximum intensity (max) and intensity and the full width at half maximum (FWHM).

RESULTS

The results are shown in the Table.

CONCLUSIONS

While ‘effective’ transducer models can well reproduce the acoustic distribution in water, they are significantly less accurate than physical representation-based models when obstacles are introduced.

<table>
<thead>
<tr>
<th>Obstacle</th>
<th>Failure [%]</th>
<th>Deviation (% normalized by tolerance) of</th>
<th></th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Position of max</td>
<td>FWHM</td>
</tr>
<tr>
<td>Water background</td>
<td>Effective model</td>
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<td>Water background</td>
<td>Physical model</td>
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<tr>
<td>Obstacle plate</td>
<td>Effective model</td>
<td>19</td>
<td>-135</td>
</tr>
<tr>
<td>Obstacle plate</td>
<td>Physical model</td>
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<td>-72</td>
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<tr>
<td>Sheep skull</td>
<td>Effective model</td>
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<td>-100</td>
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<tr>
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<td>Physical model</td>
<td>0</td>
<td>-92</td>
</tr>
<tr>
<td>Pig skull</td>
<td>Effective model</td>
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<tr>
<td>Pig skull</td>
<td>Physical model</td>
<td>0</td>
<td>-41</td>
</tr>
</tbody>
</table>

TABLE: Results of the comparison. In one case the obstacle was too close to the focus to measure the FWHM. The deviations are normalized to the chosen tolerances, i.e. values outside the range from -100 to 100 exceed the tolerance limits.
IMPACT OF THE SKULL MODEL ON SIMULATED TFUS BEAM PROFILES

The following abstract was accepted for the 40th International conference of the IEEE Engineering in Medicine and Biology Society (EMBC).
Impact of the skull model on simulated TFUS beam profiles

Cristina Pasquinelli, Hazael Montanaro, Esra Neufeld, Hyunjoo J. Lee, Axel Thielscher, Member, IEEE

Abstract — Simulations of TFUS (transcranial focused ultrasound stimulation) beam profiles are important for targeting and estimations of the US intensity distribution inside the head. Here, we demonstrate the importance of the skull model for accurate calculations of the distributions, based on systematic comparisons with water-tank measurements.

I. INTRODUCTION

Simulations play a key role in targeting and dose calculations for the design and analysis of TFUS experiments, as well as for treatment assessment and personalization. When modeling the skull as homogeneous, our simulations deviated strongly from experimental data. Here, we report initial results of a systematic study to identify the relevant parameters for the skull model that determine the acoustic exposure by TFUS. In particular, we test whether inhomogeneous material properties estimated from CT intensity data improve the correspondence between measurements and simulations.

II. METHODS

A curved single-element transducer (500 KHz) was immersed in a water tank and the pressure field measured using a calibrated hydrophone with/without precisely positioned pig and sheep skull fragments, characterized by computed tomography (CT). The measurements were compared to acoustic simulations of the corresponding setup models. The skull acoustic velocity and attenuation properties were first modeled as homogeneous bone (cortical or average of CT image-based bone property maps). Then, the acoustic properties were derived from CT intensity data as described in [1]. The sensitivity of the simulated beams to the parameters of the (linear and non-linear) mapping functions from CT to acoustic properties was tested.

III. RESULTS & CONCLUSION

Fig. 1 compares measurements and simulations. Absorption maps have been found to impact intensity but only weakly affect focus shape and pressure distributions. When modeling skull as homogeneous, focus shape strongly deviates from the measured shape. Considering CT-based skull inhomogeneity information results in much improved agreement, and optimization of the bone absorption property results in good prediction of intensity (<10% error in predicting the important transducer distance dependence). However, different absorption relations had to be used for sheep and pig skulls (otherwise, a factor 4 disagreement results). This could be the result of species specific CT-properties relationships, or of strong relationship non-linearity. Additional imaging involving reference materials is currently performed to narrow the related uncertainty. These findings demonstrate the need to better characterize the relationship between CT density and acoustic properties, in line with the important variability found in literature [1,2].

REFERENCES

APPENDIX

E

MINIATURE ULTRASOUND RING ARRAY
TRANSDUCERS FOR TRANSCRANIAL ULTRASOUND
NEUROMODULATION OF FREELY-MOVING SMALL
ANIMALS

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Miniature ultrasound ring array transducers for transcranial ultrasound neuromodulation of freely-moving small animals

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ABSTRACT

Background: Current transcranial ultrasound stimulation for small animal in vivo experiment is limited to acute stimulation under anesthesia in stereotaxic fixation due to bulky and heavy curved transducers. Methods: We developed a miniaturized ultrasound ring array transducer which is capable of invoking motor responses through neuromodulation of freely-moving awake mice. Results: The developed transducer is a 32-element, 183-kHz ring array with a weight of 0.035 g (with PCB: 0.73 g), a diameter of 8.1 mm, a focal length of 2.3 mm, and lateral resolution of 2.75 mm. By developing an affixation scheme suitable for freely-moving animals, the transducer was successfully coupled to the mouse brain and induced motor responses in both affixed and awake states. Conclusion: Ultrasound neuromodulation of a freely-moving animal is now possible using the developed lightweight and compact system to conduct a versatile set of in vivo experiments.

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Introduction

Transcranial focused ultrasound stimulation (tFUS) is a promising modality because of its competitive advantages such as focusing capability (i.e., high spatial resolution), beam steering capability, and long-term safety [1]. However, previous works on small animals have been limited to acute stimulation under various anesthetic levels (light to deep) in stereotaxic fixation due to bulky and heavy curved transducers. Here, we propose a light-weight capacitive micromachined ultrasound transducer (CMUT) ring array suitable for non-invasive brain stimulation for chronic experiments. We demonstrate the feasibility of neuromodulation using the proposed miniaturized transducer ring array in both acute and awake mouse experiments. This system enables freely-moving animal behavior studies where the effects of ultrasound neuromodulation could be observed in real-time in both acute and chronic conditions. For example, both immediate and chronic effects of ultrasound modulation on epilepsy frequency, sleep patterns, and cognitive performance could be observed using the proposed system.
Materials and methods

We designed and fabricated a ring array with an outer diameter of 8.1 mm and an inner diameter of 5.2 mm to generate a focal point at approximately 2.3 mm from the device with an immersion resonant frequency of 183 kHz (Fig. 1A, B, S1). Ring array was chosen because of the following advantages: natural focus at the center (Figure S2), larger aperture while minimizing localized skull heating, and extra room in the middle for integration with other devices. The ring array is composed of 32 elements, and each element is composed of 12 circular resonating plates (or cells) connected in parallel [24,25]. The weights of the ring array and fully-packaged array with a custom-designed printed circuit board (PCB) were 0.035 g and 0.73 g, respectively (Fig. 1C). For further information, see Supplementary Methods.

Results

Beam profile of the miniature ring array

A volumetric hydrophone scan of 5-mm wide and 10-mm long was performed with a 0.25-mm step from the center of the surface of the ring array (Figure S3). The CMUT ring array was biased at 100 DC voltage superimposed with a 183-kHz, 39.6 AC voltage. Full-Width Half-Maximum (FWHM) (i.e., focus size) of 10.13 mm² in the horizontal plane and 6.12 mm² in the vertical plane with a focus length of 2.3 mm and a maximum intensity of 50 mW/cm² (27 kPa) were observed (Fig. 1F). These measurement results were comparable to the simulated beam profile (COMSOL Multiphysics®, Burlington, MA, USA) (Fig. 1E). The intensity at the focal point increased as the AC voltage increased where a maximum intensity of 174 mW/cm² (~52 kPa) was achieved at an AC peak-to-peak voltage of 90 V (Fig. 1D). In addition, while impedance measurement in air showed a resonant frequency of ~780 kHz, Fast Fourier transform (FFT) of the measured transient pressure showed a center frequency of 183 kHz and a 3dB bandwidth of 179 kHz (fractional bandwidth of ~98%) (Figure S4, S5). Lastly, to ensure that our device does not cause a significant heating to activate neuronal activities, we measured potential temperature increase using an Agarose gel phantom and a thermocouple. We observed a temperature increase of approximately 0.1 °C after ~240 s of continuous sonication (Figure S6). Since the duration of sonication in our in vivo protocol is only 0.2 s, the temperature effect should be negligible.

MR compatibility of the miniature ring array

Since the target stimulation area is difficult to determine, non-invasive ultrasound neuromodulation could be accompanied with functional magnetic resonance imaging (fMRI). Thus, it is important for the CMUT device and the package system to be MR conditional. Here, we assessed the influence of the device with and without packaging on MR imaging quality using a 3T clinical scanner and an agar-filled spherical phantom. The device alone did not cause measurable effects on the MR images (Fig. 1H) when compared to the baseline measurement (Fig. 1G). Nevertheless, the initial measurement of the packaged device revealed clear distortions of the static magnetic field of the scanner, which were caused by the connector mounted on the PCB (Fig. 1C). Without the connector, at the power-up with DC bias voltage alone, the adverse effects of the presence of the device on the MR images has substantially decreased (Fig. 1I). In addition, while the device alone did not induce RF noise above the thermal noise floor, the noise was clearly measurable once the device was connected to the RF amplifier and DC power source placed outside of the MR cabin. The issues with the RF noise can be readily resolved using a physical filter attached to the MR cabin for future applications [26].

Acute in vivo neuromodulation of motor cortex

To confirm the functionality of the ring array, we performed in vivo acute mouse experiment without craniotomy. The CMUT array was biased at DC bias voltage of 100 V and driven with AC peak-to-peak voltage of 80 V at 183 kHz. Each stimulation trial consisted of 40-pulses of 90% duty cycle at a pulse repeat frequency (PRF) of 200 Hz, and each 4.5-ms long pulse consisted of 756 pulses of ultrasound (Fig. 2A, S7). During this trial, the total ultrasound power that was delivered (i.e., pulse intensity integral (PII)), was 0.28 mJ/cm² and spatial-peak, temporal-average intensity (Ispta) was 55.4 mW/cm². The success rate of motor responses was measured at an increasing intensity by adjusting the AC voltage. At each intensity, approximately 25 stimulation trials were conducted over 4 min and the event of 'success' and 'fail' was determined based on a threshold (i.e., 3 times the EMG noise floor) (Fig. 2B and C). For all four mice, an increase in the success rate was observed as the intensity (Ispta) increased (Fig. 2D), which was comparable to the ones demonstrated in the literature where bulky ultrasound transducer was used [3]. At an intensity of 34.1 mW/cm², the average success rate of four mice was over 70% (see Movie S1).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.brs.2018.11.007.

Three control experiments were also performed to ensure that the motor responses were not evoked due to any potential electrical leakage or buzzing sound. First, the success rate of four mice was measured when the device was biased with DC bias voltage of 100 V and AC voltage of 0 V. The observed success rate was negligible (i.e., 0−7.14%) and was significantly lower than the success rate when AC voltage of 80 V was applied with the paired t-test P value of 0.0044 (Fig. 2E). The second control experiment was performed by applying 0 DC bias voltage with an AC voltage of 80 V on one mouse. The success rate of 6.8% was observed which was much lower than the success rate when the device was fully driven (i.e., 100 VDC + 80 VAC). Thus, the evoked motor responses were not due to any potential electrical leakage between the device. The last sham experiment was conducted to evaluate artifacts due to the buzzing sound of the transducer [27,28]. While using the identical in vivo procedure and experimental setup, the packaged device was placed between the metal slits upside down. When the device was flipped, the success rate was 3.44% which was significantly smaller than that observed during the normal stimulation. When there was no device attached to the system, we have observed the success rate due to the spontaneous movements as high as 10.7%. Thus, the effect of potential artifacts due to the buzzing sound should be negligible.

In vivo neuromodulation of motor cortex of awake animals

For neuromodulation of freely-moving mice, compact interface and package are essential. Specifically, design and implementation of three components were required: head fixture, collimator, and electric rotary joint to provide the input voltage to the CMUT ring arrays (Figure S8). Using this system, we successfully demonstrated the transcranial ultrasound neuromodulation of a freely-moving mouse where a success rate of 100% over 10 trials was observed when the device was fully driven (see Movie S2). We observed that the transducer coupled to the head did not cause impairment in its ability to walk, feed, and groom (see Movie S3). The stimulation was conducted approximately after 3 h from the surgery. Although no EMG signals were recorded due to limited surgery techniques, the stimulation was visually recorded. After 7 days of the implantation,
when additional ultrasound coupling gel was applied, we visually observed successful modulation over 10 trials. Not only our proposed interface system permitted stimulation of freely-moving animals over 7 days, the device was replaceable and reusable after the experiments. Lastly, we observed no significant tissue damage such as vascular hemorrhage and neuronal necrosis compared to that of the control mice (Figure S9). This result suggests that CMUT ring array produced no significant microscopic damage to the mouse cortex, which is similar to the results of single-element bulky transducers [29].

Supplementary video related to this article can be found at https://doi.org/10.1016/j.brs.2018.11.007.
Discussion

By devising a miniature ultrasound transducer array, we have demonstrated the possibility of performing transcranial ultrasound neuromodulation during both acute and awake states. Although more efforts are required to investigate the mechanism of ultrasound neuromodulation (indirect or direct) [19,20], the proposed system provides the same functionalities as that of commercial bulky transducers but with a new capability of enabling freely-moving experiments. Although the intensity of our device was relatively low compared to the previous works (~10 W/cm²), we demonstrated that the intensity was sufficient for neuromodulation of motor cortex without craniotomy. To achieve a higher intensity while maintaining the resonant frequency, a thicker silicon circular plate with a smaller radius could be used [30]. In the succeeding developments, there are several limitations that needs be overcome. First, we have only tested our system on wild-type mice. We plan to apply our system to different animal disease models to demonstrate biologically meaningful therapeutic effects. Second, the fabricated device was in the form of an array, and thus, when interfaced with beam-forming circuits, dynamic focusing could be achieved to target different locations within the brain without relocating the device. For beam-steering, the individual element of the ring array must be also interfaced with a separate circuitry using multichannel driving systems such as commercially systems (e.g., Verasonics©), custom-designed ICs, or multi-channel RF amplifiers. However, because of the inherent beam shape of the ring array, we still achieved a narrow single-focus along the stimulation axis which contributed to the simplification of our setup. Thus, future work includes interfacing our device with beam-forming circuitries for dynamic focusing and beam-steering.

Conflicts of interest

None declared.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2018.11.007.

Fig. 2. Ultrasound neuromodulation of motor cortex using CMUT ring array. (A) Schematics of the trigger waveform delivering 40 pulses of 756 ultrasound pulses. The total power delivered through this waveform is described in different metrics. (B) Schematics illustrating ‘success’ events determined when the EMG power was larger than 3 times the noise floor. (C) Example of recorded raw EMG signals at the incident of (i) ‘fail’ and (ii) ‘success’ events. (D) Success rate measured over approximately 25 stimulation trials for 4 mice at varying ultrasound intensities (ISPPA) controlled by AC voltages. (E) Success rate of a control case when 100 DC voltage (VDC) was applied with 0 VAC.
References


Miniature ultrasound ring array transducers for transcranial ultrasound neuromodulation of freely-moving small animals

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SUPPLEMENTARY MATERIALS

Methods

Process flow for fabrication of CMUT ring array
A silicon wafer with high conductivity was used as the starting wafer, which served as the bottom electrodes (see Figure S1). The 1.85-µm-tall vacuum cavities were defined through the double oxidation process. A 2-µm-thick oxide was first grown through wet oxidation at 1100°C. Then, the cavities were etched through the photoresist mask layer using Buffered Oxide Etch (BOE) 6:1, and a 250-nm-thick second oxide layer was grown (wet oxidation at 1100°C) as an insulation layer. Because the oxidation process is highly controllable, cavities with uniform height were achieved. The prepared substrate was bonded to a Silicon-on-Insulator (SOI) wafer with a 2-µm-thick top silicon layer through direct wafer bonding under the vacuum environment. The bonded wafer was annealed at 900°C to maximize the bonding strength. The handling substrate and Buried-Oxide (BOX) layer of the bonded SOI wafer were then removed through the Chemical Mechanical Polishing (CMP) and wet etching in BOE 6:1, respectively. The element shape consisting of 12 resonating cells was then defined by etching the top silicon through Reactive Ion Etching (RIE). In addition, the oxide layer was wet etched using BOE 6:1 to access the bottom substrate as the ground electrode. 10-nm-thick chromium (Cr) and 250-nm-thick gold (Au) layer were then evaporated on top to provide electrical access and wet etching using the relevant metal etchants. Finally, through-wafer etching was performed from the backside of the wafer to define the ring array shape using the Deep Reactive Ion Etching (DRIE).

Device packaging
The successfully fabricated ring array was attached to a customized-designed Printed Circuit Board (PCB) using a non-conductive 5-min epoxy. Ground and signal pads of each element were electrically connected to the PCB using gold wire-bonding. Because no beamforming was performed in this work, all the elements were shorted to the common signal and ground pads on the PCB. The two corners of the PCB were rounded to
minimize the unnecessary area around the ring array on mouse head (Figure 1C). A socket was attached at the end of the PCB to provide DC and AC voltage to the ring array. Any shortage or opening of the wire-bonding was confirmed through the visual inspection and the measurement of electrical impedance of the CMUT ring array. The gold wire-bonds were then covered with a small amount of the non-conductive 5-min epoxy to prevent any physical damage. No passivation was performed on our device because the beam characterization and in vivo experiments were both performed in oil. In addition, no matching layer was required because CMUT composed of thin silicon plates exhibits a low mechanical impedance and thus the mismatch between the device and the fluid medium is low [1].

**Characterization of electrical input impedance**

The electrical input impedance of each element in the ring array was measured using an impedance analyzer (Model E4990A, Agilent Technologies Inc., CA, USA) and a probe station. A high-DC power supply (PS 310, Stanford Research Systems, CA, USA) was used to supply DC voltage to the device, which was superimposed by a 50-mV AC voltage of varying frequencies supplied by the impedance analyzer. DC and AC voltages were superimposed through a bias tee which prevents any leakage current to be passed on to the input of the impedance analyzer. The impedance magnitude and phase were measured over a frequency range (150 kHz ~ 1 MHz) at an increasing DC bias voltage from 50 V to 100 V (Figure S3).

**Ultrasound stimulation protocol**

A combination of a high DC bias voltage (PS310, Stanford Research Systems Inc., Sunnyvale, CA) and an AC voltage was applied to drive the CMUT ring array (Figure S2). The AC voltage was generated through two function generators (33220A, Agilent Technologies, CA, USA). The first function generator controlled the pulse repetition frequency of the second function generator, which generated an AC voltage at a resonant frequency of the device (Figure 2A). The signal from the second function generator was amplified by a 47 dB RF amplifier (5312F, OPHIR RF, CA, USA) to drive CMUT with the maximum amplitude of AC signals.
The DC bias voltage and the AC voltage were superimposed through a bias-tee with a resistor and a DC-blocking capacitor through a customized printed circuit board (PCB). To generate the ultrasound, the DC bias was increased slowly to 100 V, and the trigger to the first function generator was sent from the computer. These trigger signals were synced with the measurement of EMG signals to accurately monitor the success rate through the custom-designed MATLAB code.

**Ultrasound beam characterization**

The beam profile of the packaged CMUT ring array was measured in an aluminum and acryl-based custom-designed tank filled with soybean oil (Figure S2). Soybean oil was used to prevent the electrical shortage and to provide a medium with similar acoustic impedance (~1.36 MRayls) to that of a brain (1.60 MRayls) [2]. A 0.5mm diameter needle-type hydrophone (NH0500, Precision Acoustics, UK) was positioned above the ring transducer in the axial direction aligned to the center of the ring array. The amplified output of the hydrophone was measured through a digital oscilloscope (DSOX2022A, Agilent Technologies, USA). The hydrophone was moved using a custom-coded stepping-motor-controlled positioning system (Science Town, Korea) with movement resolution of 0.25 μm. The acoustic amplitude and phase were measured across a 10 × 20 mm² area at 0.25-mm intervals in the lateral direction and 0.25-mm intervals in the axial direction. The points in the map were plotted every 0.25 mm step for both axes.

**Evaluation of MR compatibility**

MR conditionality was tested in a 3 T MRI scanner (Prisma, Siemens Healthcare, Erlangen, Germany) equipped with a 64-channel head receive coil. A 17-cm-diameter spherical phantom filled with doped agar and exhibiting T1 relaxation and RF conductivity similar to brain tissue was used [3]. We assessed MR conditionality using three sequences: In order to test for distortions of the static magnetic field, a double-echo gradient echo sequence was employed to create B0 maps (TR/TE1/TE2=520/4.92/7.38 ms, FA 60°, slice thickness 2 mm, 49 slices, in-plane resolution 1.5 × 1.5 mm², FoV 192 × 192 mm², coronal slice orientation).
The emission of radio frequency (RF) noise was measured using a Siemens service sequence, which records the RF spectrum in a range between +/- 250 kHz around the center frequency of the scanner. Finally, gradient-echo echo planar images (GE-EPI; TR/TE=1430/30 ms, FA 70°, slice thickness 1.5 mm, 60 slices, in-plane resolution 1.5 x 1.5 mm², FoV 210 x 210 mm², 20 volumes, multi-band factor 2, horizontal slice orientation) were acquired to test specifically the compatibility of the device with functional MRI [4].

Testing was done in several successive steps: In order to establish a baseline, the sequences were first run without the device in place. The measurements were then repeated with (i) only the ring array attached to the phantom, (ii) the full device attached, (iii) the device attached, but without the connector (Figure 1C), (iv) the connected device attached, and finally (v) the connected and powered up device attached. The ring array and device was placed on top of the phantom and gently fixed using sticky tape. Among six tests performed, we show three representative images of baseline, (i), and (v) (Figure 1G-I). During tests iv and v, the device was connected via the bias tee to an RF amplifier (240L power amplifier, E&I, USA) and a DC current source (IPS 2303, RS Pro, Corby, UK) placed outside the RF cabin of the scanner. During test v, the RF amplifier received a continuous sinusoidal signal from a function generator (33500B series waveform generator, Keysight technologies, USA) with a center frequency of 120 kHz and an amplitude of 100 mVpp. A gain of 50 dB was set at the RF amplifier, and the DC supply provided 30 V. For analysis, the B0 maps were processed using fsl_prepare_fieldmap from the FSL toolbox (www.fmrib.ox.ac.uk/fsl) to unwrap the phase images and create fieldmaps scaled in rad/s.

**In vivo ultrasound stimulation**

Before proceeding with *in vivo* experiments, the PCB and the device were passivated in a polyethylene film and filled with oil to avoid any unexpected electric shock to a mouse. A total of 4 C57Bl/6J mice (male, 4–6 weeks old) were employed in this study. All experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee at the Korea Advanced Institute of Science and
Technology (KA2017-28). At the beginning of each experiment, the mouse was anesthetized with 1.2% Avertin solution at a dosage rate of 0.25 ml/10 g body weight. Ophthalmic ointment was used to protect eyes from drying. The fur over mouse’s head and the area where the EMG electrodes were to be inserted was cut. Then the mouse’s head was gently immobilized using a stereotaxic apparatus (Harvard Apparatus, Holliston, MA, USA). For the device alignment, we fixed the corner of the PCB to the vertical pole attached to the stereotaxic frame. The distance between the corner of the PCB and the center of the CMUT ring array was accurately known through the PCB layout. The vertical pole was first aligned to the bregma and adjusted by the known distance in x and y directions so that the center of the ring array was aligned to the bregma. Then, the vertical pole was moved by the target stereotaxic coordinate of AP of 0.5 mm and ML of 1.5 mm. We adjusted the thickness of the packaging layer of the PCB so that the maximum intensity of the beam profile in the axial direction was targeted at DV of 0.5 mm. To quantify the motor response, fine-wire stainless steel electrodes were inserted into the mouse’s forepaw muscle and trapezius muscle. Approximately 25 stimulation trials were conducted over a time window of 4 min. It is important to distinguish the evoked motor responses due to the stimulation from that of the spontaneous movements. Thus, the minimum interval between the stimulation trials was set to 8 s. Moreover, if the EMG signal increased due to any movements within 1.5 s before the sonication, the scheduled trigger was skipped and delayed for 1.5 s until the EMG signal was quiet (Figure S5).

**Chronic stimulation setup**

We devised a new head fixture that not only firmly secured the device on the mouse head and but also offered the flexibility to replace the device after the fixation. The fixture consisted of two metal slits where the CMUT-interfaced PCB was placed in between. The bottom slit was fixed on the skull instead on the skin using dental cement to minimize the movement of the fixture. Since only the bottom slit was secured on the mouse skull using dental cement, the PCB board with the CMUT ring array was readily removable and replaceable. The flexibility of moving the device after fixation is important as it allowed evaluation of the
transducer functionality during the experiments. The space between the CMUT ring array and the skull must be interfaced with a coupling medium to transmit ultrasound efficiently. In addition, during the experiments, this coupling medium should not be dried up. Thus, for this work, we chose oil as an insulating and coupling medium which were encapsulated through a thin plastic wrap. A small amount of ultrasound coupling gel was applied to fill any space between the brain and encapsulated device. Lastly, we used an electric rotary joint (SRC012-6-H, HPM & ET Co. Ltd, China) to provide DC and AC voltage to the CMUT ring arrays and to transmit the EMG signals back to the recording system.

**EMG recordings and processing**

The EMG signals from the muscles were amplified by a gain of 1000, bandpass-filtered (0.1 Hz ~100 Hz), and digitized with a 1-kHz sampling rate through a bio-potential acquisition device (RHD2000, Intan Technologies, Los Angeles, CA, USA). The recorded EMG signals were compared to the trigger signals and processed using a custom-designed code in MATLAB to mark the stimulation as ‘success’ or ‘fail’ (MathWorks®, Natick, MA, USA). The as-recorded signals were used directly without the rectification for the analysis because the increase in the EMG signals was sufficiently large in one direction when successfully stimulated. Moreover, to minimize the probability of counting spontaneous movements as ‘success’, the following three conditions were applied. First, we calculated the absolute average of the EMG signals, \( P_{\text{quiet}} \) measured over 20 s at the beginning of every experiment when there was no apparent movement of a mouse. Thereafter, the absolute average of every 60-ms of the EMG signals, \( P_{\text{60ms}} \) was compared to \( P_{\text{quiet}} \). If \( P_{\text{60ms}} \) was larger than \( P_{\text{quiet}} \) by 1.2 times, we assumed there was a spontaneous movement and waited 1.5 s for the next evaluation of the EMG signals. Only when \( P_{\text{60ms}} \) was smaller than \( P_{\text{quiet}} \), the trigger for the ultrasound stimulation was activated. Second, for every trigger, we waited sufficiently long (8 s) to allow each stimulation to be independent. After 8 s, the EMG signals were compared again against \( P_{\text{quiet}} \) following the first protocol. Third, upon stimulation, only if a significant change in the EMG signals (larger than 3 times the noise level) was observed between 0.06 and 0.4 s from the trigger, the event was considered a ‘success’.
H&E staining of brain slices

Mouse brain fixation and paraffin block mounting were performed as standard protocol. Briefly, Mice received pulsed ultrasound for 30 min in the same manner as described above was compared to mouse not receiving ultrasound, but with the same fixed condition. After 30 minutes of a recovery period, each stimulated or unstimulated mouse was sacrificed with isoflurane, and transcardial fixation with phosphate-buffered 4% paraformaldehyde was performed. We carefully harvested the sacrificed mouse brains and postfixed them in 4% paraformaldehyde solution for overnight. After decalcification for 13 hours, paraffin blocks were mounted. 4-µm-thick coronal slices of mouse brains were prepared using a microtome (Leica RM 2135). Acquired brain sections were stained with hematoxylin and eosin. We evaluated gross impairment findings (vascular hemorrhage or neuronal necrosis) in the Hematoxylin-Eosin stained brain section. The criteria of brain tissue damage were described in the previous study [5]. We confirmed whether hemorrhage or extravasation of fluid and red blood cell occurred due to an injury of the cortex. In addition, to observe mineralization pattern, we identified acute or late-stage necrosis. All visual inspections were done using Olympus BX51 microscope (Olympus, Tokyo, Japan) equipped with a CCD camera and computer-assisted image analysis with DP2-BSW at various magnifications.
Supplementary Figures

Figure S1. Fabrication process of CMUT ring array.

a, First oxidation

b, Cavity definition

c, Second oxidation

d, Fusion bonding

e, Remove SOI substrate & BOX layer

f, Si membrane and ground patterning

g, Metal evaporation & patterning

h, Release

Silicon
Silicon oxide
Cr/Au
Figure S2. Field II simulation of ultrasound beam profile generated from different types of arrays: (i) 1D array (XY plane), (ii) 1D array (YZ plane), (iii) piston (circular), (iv) 2D array (square), (v) ring array, and (vi) piston concave circular aperture. The color scale represents the relative intensity of beam with the strongest shown in red.
Figure S3. Schematics of experimental setup to measure the beam profile of the CMUT ring array.
Figure S4. (A) Electrical input impedance of one element biased at varying DC voltages. (B) Parallel resonant frequencies of the elements in the ring array under the DC bias voltage of 100 V.
Figure S5. (A) Impulse response of the device to an input of 1-ns 10 Vpp pulse. (B) Frequency domain of the impulse response.
**Figure S6.** Temperature change of an agarose gel measured using a thermocouple placed at the focal spot over 30 s of continuous pulsation using the CMUT ring array. The table summarizes the changes in the temperature using commercial transducers noted in other works.
Figure S7. Experimental setup for the measurement of success rate. The PCB packaged with the CMUT is mounted on the mouse head. PRF is controlled by the first function generator. At the ON trigger, the second function generator inputs an AC waveform to generate an ultrasound waveform consisting of 756 pulses and the LED is simultaneously turned on. Throughout the experiments, the EMG signals and videos are recorded in real-time.
Figure S8. Schematic drawings of the chronic interface showing the oil encapsulation of the device, two-metal slits head fixture, and electric rotary joint.
Figure S9. Histology of brain slices. (A) H&E stain images of a mouse brain slice when the mouse brain was sonicated using CMUT ring array driven with maximum power. (B) H&E stain images of a mouse brain slice when the mouse was fixed in a stereotaxic frame with CMUT ring array on top, but no ultrasound was delivered.
Movie S1.

Acute neuromodulation using the fabricated CMUT ring array.

Movie S2.

Neuromodulation of freely-moving animals using the CMUT ring array after 7 days.

Movie S3.

Movement of a mouse with the miniature transducer coupled to its head showing no impairment in walking, feeding, and grooming.

DATA AND SOFTWARE AVAILABILITY

The custom MATLAB scripts used to estimate the success rate can be found in https://www.dropbox.com/sh/79qe1vrmhul1g0h/AABBywp5cP3B4Yqk1Ke1ViBha?dl=0.

AUTHOR CONTRIBUTIONS

H.K. and H.J.L. conceived and designed the experiments and wrote the manuscript. H.K. fabricated and characterized the CMUT ring array and performed the in vivo mice experiments. S.K. performed COMSOL simulations and measured the beam profile of the CMUT devices. N.S.S. and J.H.L. performed H&E staining the brain slices. A.T. and C.P. conducted the MR experiments.

REFERENCE


