

**Towards precision non-invasive
neuromodulation:
modelling and evaluation of transcranial
focused ultrasound systems**

Han Li

Doctor of Philosophy

University of York

School of Physics, Engineering and Technology

May 2026

Abstract

Transcranial focused ultrasound (tFUS) is being investigated as a non-invasive neuromodulation approach with the potential to reach deep brain structures with greater spatial selectivity than many established non-invasive techniques. A growing literature suggests that ultrasound can influence neural systems across multiple biological scales, from ion-channel and synaptic effects in cellular preparations to functional and behavioural responses in animals and humans. However, the interpretation of such effects depends critically on knowing the acoustic field delivered to the intended neural target. This remains challenging because the skull can attenuate, refract and distort the transmitted acoustic field, complicating both treatment planning and the interpretation of neuromodulatory outcomes. This thesis examines the physical and methodological basis of transcranial focused ultrasound delivery. It first establishes a framework for acoustic source characterisation and intracranial field measurement. Using this framework, the thesis shows that intracranial delivery depends not only on frequency and skull morphology, but also on transducer-skull spacing. Building on these experimental observations, an analytical model is developed for rapid estimation of transcranial insertion loss. Multiple skull-modelling strategies in k-Wave are then benchmarked against measurements, showing that modelling choices can affect predicted pressure transmission and focal displacement. Finally, a preliminary in-vivo feasibility study demonstrates that tFUS delivery can be integrated with rat hippocampal electrophysiology while preserving interpretable electrophysiological recordings.

Declaration

I, Han Li, hereby declare that this thesis is a presentation of original work and that I am the sole author. This work has not previously been presented for a degree or other qualification at this University or elsewhere. All sources are acknowledged as references.

I acknowledge that I received assistance from ChatGPT for proofreading, in line with the University of York Policy on Transparency in Authorship in PGR Programmes.

Publications arising from this work, for which the author contributed as first or co-first author, are listed below.

Li, H., Barnard, I., Halliwell, T., Zhang, X., Melzer, A., & Huang, Z. (2025). Effects of skull properties on continuous-wave transcranial focused ultrasound transmission. *The Journal of the Acoustical Society of America*, 157(4), 2336–2349. <https://doi.org/10.1121/10.0036344>

Li, H., Zhang, X., Halliwell, T., Wang, N., & Huang, Z. (2025). A multi-layer transcranial focused ultrasound model for neuromodulation procedure planning and insertion loss estimation. *Physics in Medicine & Biology*, 70(21), 215024. <https://doi.org/10.1088/1361-6560/AE1543>

Wang, N., Li, H., Liao, J., Halliwell, T., & Huang, Z. (2026). Predicting transcranial ultrasound insertion loss using skull CT: A deep learning approach. *Ultrasonics*, 163, 107976. <https://doi.org/10.1016/J.ULTRAS.2026.107976>

Li, H., Horner, A. J., Zhang, X., & Huang, Z. (2026). Experimental Validation of Skull Acoustic Modelling Strategies for Transcranial Focused Ultrasound Simulation: A Cross-Comparison Study. <https://arxiv.org/pdf/2606.09497>

Acknowledgments

I would like to express my deepest gratitude to my supervisor, Professor Zhihong Huang, for her insight, guidance, and steadfast support throughout my research. Her clear sense of direction, generosity in sharing knowledge and resources, and constant encouragement have been invaluable to the progress of this work. Thank you for everything.

I would also like to acknowledge the collaborators with whom I have had the pleasure of working. My sincere thanks go to Dr. Chunhui Li, Dr. Isla Barnard and Dr. Tom Gilbertson for generously sharing their ideas, and time. I am also grateful to Mr. Tyler Halliwell for his willingness to provide human anatomy resources, and to Dr. Stephen Martin for opening the door of electrophysiology to me.

To all of my colleagues in Zhihong's team, thank you for the long days and late nights, for the enjoyable moments we spent together, and for the support, encouragement, and research passion we shared. Working alongside you has broadened my horizon in many ways.

My deepest appreciation goes to my family, who have always been my backbone. Thank you for supporting me in every aspect of life and for giving me the strength to keep moving forward. To my life partner, Xinyu, thank you for walking beside me through bitterness and sweetness alike.

Finally, I would like to thank both the University of York and the University of Dundee for educating and supporting me from my undergraduate studies to my PhD, and for allowing me to grow not only as a researcher, but also as a person. I am deeply thankful for the opportunity to develop my expertise through this journey.

Contents

Abstract.....	2
Declaration.....	3
Acknowledgments	4
List of Figures.....	8
List of Tables.....	12
List of abbreviations and symbols	13
1. Introduction.....	16
1.1. Motivation	16
1.2. Aims and structure	17
2. Literature review.....	19
2.1. Brain disorders	20
2.1.1. Major depressive disorder.....	20
2.1.2. Current treatment approaches	22
2.2. Neuromodulation.....	24
2.2.1. Electrical modalities.....	25
2.2.2. Magnetic stimulation	30
2.3. Focused ultrasound neuromodulation	32
2.3.1. Biophysical mechanisms.....	33
2.3.2. Early translational studies	35
2.3.3. Cortical neuromodulation studies	35
2.3.4. Subcortical neuromodulation studies	41
2.4. Ultrasound transmission through the skull.....	43
2.4.1. Role of the cranial bone	44
2.4.2. Targeting	46
2.4.3. Characterisation	47
2.4.4. Computational modelling.....	48
2.4.5. Monitoring and validation.....	48
3. Acoustic source characterisation	50
3.1. Piezoceramics.....	51
3.1.1. Linear constitutive relations.....	51

3.1.2.	Reduced thickness-mode model	53
3.1.3.	Resonant behaviour	54
3.1.4.	Pressure gain estimates	56
3.2.	Energy characterisation	57
3.2.1.	Momentum flux expression	57
3.2.2.	Power measurement	60
3.2.3.	Pressure field measurement	62
3.2.4.	Acoustic holography	66
3.3.	Discussion	69
4.	Transcranial characterisation	73
4.1.	Introduction	74
4.2.	Skull specimen	75
4.2.1.	Historical and Thiel-embalmed skulls	75
4.2.2.	Skull segments structural characterisation	77
4.3.	Transcranial energy insertion loss	80
4.3.1.	Field mapping	80
4.3.2.	Pressure and power loss	82
4.4.	Transcranial field distortion	87
4.4.1.	Phase error	87
4.4.2.	Focus expansion	91
4.4.3.	Focus shift	93
4.5.	Transcranial energy uncertainties	93
4.5.1.	Angulation effect	93
4.5.2.	Wave superposition effect	95
4.6.	Discussion	97
5.	tFUS analytical solution	101
5.1.	Introduction	102
5.2.	Derivation of skull properties	103
5.2.1.	Longitudinal sound speed	103
5.2.2.	Mass density and attenuation coefficient	105
5.2.3.	Dynamic three-layer segmentation	105
5.2.4.	Target-based ray tracing and skull-characteristic mapping	107
5.2.5.	Full skull characteristic maps	108
5.2.6.	Insertion angle map	109
5.3.	Analytical model of transcranial transmission	111

5.3.1.	Monochromatic plane-wave approximation	111
5.3.2.	Six-layer propagation framework	111
5.3.3.	Full-skull transmission efficiency estimation	115
5.4.	Model validation	116
5.4.1.	k-Wave solver	116
5.4.2.	Benchmark in simplified layered geometry	117
5.4.3.	Experimental validation setup.....	119
5.4.4.	Cross-validation in ex-vivo skulls	120
5.5.	Validation results.....	123
5.5.1.	Frequency-dependent insertion loss prediction	123
5.5.2.	Oscillatory behaviour and spacing uncertainty.....	126
5.6.	Discussion	127
5.6.1.	Comparison with other bone modelling strategies.....	129
5.6.2.	Practical implications for transducer allocation.....	132
6.	Full-wave modelling and in-vivo evaluation.....	134
6.1.	Introduction	135
6.2.	Modelling framework.....	136
6.2.1.	Skull specimens	136
6.2.2.	Skull modelling strategies.....	137
6.2.3.	Experimental setup.....	139
6.2.4.	Evaluation metrics	139
6.3.	Model performance	141
6.3.1.	Representative case study	141
6.3.2.	Grouped field comparison.....	142
6.3.3.	Model-specific error and specimen effects	148
6.4.	Discussion	150
6.5.	In-vivo evaluation	152
6.5.1.	Rat intracranial acoustic characterisation	153
6.5.2.	Electrophysiology and stimulation configuration.....	155
6.5.3.	Preliminary physiological observations	158
6.5.4.	In-vivo feasibility and limitations	160
7.	Conclusion	162
7.1.	Summary	163
7.2.	Future work	167
7.3.	Conclusion.....	169

APPENDIX A	171
APPENDIX B	175
APPENDIX C	178
APPENDIX D	180
APPENDIX E	183
APPENDIX F	184
Bibliography	185

List of Figures

Figure 2-1 Schematic illustration of astrocyte–neuron interactions relevant to depressive pathology.....	22
Figure 2-2 Comparative overview of major neuromodulation modalities.....	25
Figure 2-3 Overview of focused ultrasound neuromodulation mechanisms and stimulation parameters	33
Figure 2-4 Anatomical and acoustic factors governing transcranial ultrasound transmission through the human skull	44
Figure 3-1 Coordinate axes and poling direction for an infinitesimal piezoelectric element.....	52
Figure 3-2 Piezoceramic surface displacement measurement setup using LDV.	55
Figure 3-3 Measured surface displacement in three transducers with centre frequencies (f_c) of 220 kHz, 680 kHz and 1000 kHz. The peak displacements occurred at operating frequencies (f_o) of 226 kHz, 690 kHz and 1027 kHz, respectively.....	55
Figure 3-4 Representative radiation-force measurement setup.	61
Figure 3-5 Electrical drive voltage-to-acoustic power conversion curves.....	62
Figure 3-6 Relationship between hydrophone tilt angle and received signal amplitude under 650 kHz sonication.	63
Figure 3-7 Acoustic scanning system setup.....	64
Figure 3-8 Near-field acoustic maps of the 220 kHz, 680 kHz, and 1000 kHz transducers acquired with step sizes of 1.0 mm, 0.5 mm, and 0.25 mm.....	65
Figure 3-9 Comparison of acoustic power measured by the RFB and by hydrophone integration for the 650 kHz transducer.....	66

Figure 3-10 ASM-based plane propagation of the ideal k-Wave-generated bowl source and the measured signal, viewed at the source aperture plane.	67
Figure 3-11 Source-surface acoustic pressure reconstructed by backward propagation of the measured holograms	68
Figure 4-1 CT density distribution of a representative historical skull specimen that scanned in both dehydrated and rehydrated conditions.	76
Figure 4-2 A representative skull with RoIs details. Two representative regions were chosen for the following analysis.	78
Figure 4-3 Experimental setup of the transcranial acoustic field measurement system.	81
Figure 4-4 Skull thickness versus the normalised transcranial peak pressure level (dB), power level (dB) at three frequencies.	84
Figure 4-5 Skull SDR versus the normalised transcranial peak pressure level (dB) and power level (dB).	85
Figure 4-6 Acoustic field aberration analysis for the representative regions S1 and S2	88
Figure 4-7 Skull RoI RoC versus the normalised focal area (F) at three frequencies. The focal areas were normalised to the free-field reference- a larger ratio indicates a blurred focus, and a smaller ratio indicates a sharper focus.....	92
Figure 4-8 Acoustic field visualisation of the 650 kHz transducer beam profile in free-field and with the presence of a representative skull	94
Figure 4-9 Pressure variation at the intracranial focus along with the increase of the transducer and representative skull.....	96
Figure 5-1 Representative time-advance measured with a skull sample compared with in free-field water condition.....	104
Figure 5-2 A representative historical skull segment.....	106
Figure 5-3 A representative CT hemi-sected skull volume.....	107
Figure 5-4 (a-c) Skull bulk thickness map, skull SDR map, and skull bulk density and sound speed map in respect of the Thalamic target.	108
Figure 5-5 (a-c) The outer table, diploë, and inner table layer thickness, density and speed of sound characteristics.....	109
Figure 5-6 Ray insertion angle relative to the brain target.	110

Figure 5-7 (a) A representative transducer arrangement relative to the brain target and the corresponding insertion direction.....	112
Figure 5-8 Ultrasound pressure transmission efficiency across the skull surface at four typical frequencies. Transducer layer was not taken into account.....	115
Figure 5-9 (a) The original source surface layer ($D = 60$ mm, $RoC = 75$ mm). (b and c) The source signal mask with BLI number set to 0.9 and 0.05 (default), respectively.	117
Figure 5-10 Axial focal gain when simulating with a piezo layer. The simulated results (SIM) were compared with the O'Neil analytical solution at 250 kHz, 500 kHz, 750 kHz and 1000 kHz.	117
Figure 5-11 (a) k-Wave simulation domain with the mediums sound speed setup. ..	118
Figure 5-12 Calculated (ANA) and simulated (SIM) normalised peak pressure level across 100 kHz to 1000 kHz frequency.	119
Figure 5-13 Signal amplitude measured using 1 mm hydrophone in free-field, frequency sweep from 100 to 290 kHz using 220 kHz transducer, and 300 kHz to 1000 kHz using 680 kHz transducer.	120
Figure 5-14 Ultrasound insertion angle map with transducer placement of S1 to S20. This angle is averaged over the inner and outer skull surface angle, and prior to taking ultrasound emission angle into account.	121
Figure 5-15 Measured, simulated and analysed pressure insertion loss levels of 20 skulls' RoI.....	124
Figure 5-16 (a) Group insertion loss averaged over 20 skulls' RoI.....	125
Figure 5-17 (a) Average insertion loss uncertainties caused by varying transducer-skull spacing over 20 skulls RoI.....	127
Figure 6-1 Skull specimens property maps.....	137
Figure 6-2 A representative transducer-skull fixture design.	139
Figure 6-3 Simulated focal field with a piece of RoI at S2 frontal bone at 220 kHz, 680 kHz and 1000 kHz	141
Figure 6-4 Outputs from the five models of a representative RoI at S2 frontal bone and its corresponding simulated cross-sectional focal field at 220 kHz, 680 kHz and 1000 kHz, compared with the corresponding measured fields.	142

Figure 6-5 Measured (MEA) and simulated intracranial peak pressure loss across 19 RoIs from seven skull specimens at 220 kHz, 680 kHz and 1000 kHz frequencies. T	143
Figure 6-6 Measured (MEA) and simulated intracranial intensity (over -6dB cross-sectional beam area) loss across 19 RoIs from seven skull specimens at 220 kHz, 680 kHz and 1000 kHz frequencies.	144
Figure 6-7 Hologram propagated from 2D measurement and simulated intracranial acoustic volumetric coverage (over -6dB) across 19 RoIs from seven skull specimens at 220 kHz, 680 kHz and 1000 kHz frequencies.	144
Figure 6-8 Hologram propagated from 2D measurement, and simulated intracranial peak pressure distance deviation to their free-field focus across 19 RoIs from seven skull specimens at 220 kHz, 680 kHz and 1000 kHz frequencies.	145
Figure 6-9 (a): 650 kHz FUS transducer integrated with cone, cone is filled with degassed water; and its 3D acoustic focal field.	154
Figure 6-10 (a): schematic stimulation experimental setup and the delivered US waveform. (b): the placement of stimulation and recording electrodes. (c): The representative stimulation and recording brain targets in the dorsal view. (d) the coronal view of the recording site of the DG. The graphs were hand drawn using Adobe Illustrator.	157
Figure 6-11.(a): Average pp-DG fEPSP waveform during baseline (control) and active tFUS stimulation. Shading indicates waveform variability across trials within each epoch. Dashed lines show the PS amplitude definition. (b): PS amplitudes across pre-tFUS, during tFUS and post-tFUS epochs, results were normalised to the pre-tFUS mean.	159
Figure 6-12 (a): Representative LFP segments from pre, during and post tFUS epochs. (b): power spectral density (PSD) for each epoch.	160
Figure D -3 Skull density map of S1 to S20	182

List of Tables

Table 3-1: Elastic-electric constants of PZ54 and DL-47, compiled from Qiu's Thesis and manufacturer data (Qiu, 2014).	52
Table 3-2: Comparison of measured hydrophone sensitivities at 650 kHz with manufacturer reference values.	70
Table 4-1 The mean and standard deviation of skull region-specific parameters over all 88 RoIs tested.....	80
Table 4-2 Average transcranial acoustic pressure and power insertion loss from historical and Thiel RoIs.	82
Table 4-3 Average transcranial field phase errors of the three frequencies from historical and Thiel RoIs.....	91
Table 4-4 Average transcranial field expansion ratio of the three frequencies from historical and Thiel RoIs.	93
Table 4-5 Average transcranial focus shift of the three frequencies from historical and Thiel RoIs.....	93
Table 5-1 Average thickness evaluation of three historical calvaria by external calliper measurement (MEA) and ray-tracing CT analysis (ANA).	108
Table 5-2 Peak pressure transmission ratio comparison with other studies. Literatures unspecified to cycles of burst, skull thickness and density, or transducer characteristics.	131
Table 6-1 Measured and simulated intracranial acoustic field results at three frequencies, report as median and IQR (in []).	147
Table 6-2 Average intracranial errors of simulated results using 5 skull modelling methods compared to the measurement at 220 kHz, 680 kHz and 1000 kHz.	148
Table 6-3 Pressure and intensity errors in comparison to different sample conditions.	149

List of abbreviations and symbols

Acronyms

AP	Action potential
A-P	Anteroposterior
ASM	Angular spectrum method
BBB	Blood–brain barrier
BDNF	Brain-derived neurotrophic factor
BLI	Band-limited interpolant
BOLD	Blood-oxygen-level-dependent
CA	Cornu ammonis
CT	Computed tomography
dACC	Dorsal anterior cingulate cortex
DBS	Deep brain stimulation
DC	Duty cycle
DG	Dentate gyrus
DLPFC	Dorsolateral prefrontal cortex
DRN	Dorsal raphe nucleus
DV	Dorsoventral
ECT	Electroconvulsive therapy
EEG	Electroencephalography/electroencephalogram
EMG	Electromyography/electromyogram
fEPSP	Field excitatory postsynaptic potential
FEF	Frontal eye field
FUS	Focused ultrasound
FWHM	Full width at half maximum
HU	Hounsfield unit
IQR	Interquartile range
ISI	Inter-sonication interval
LDV	Laser Doppler vibrometer
LFP	Local field potential
LGN	Lateral geniculate nucleus
LTP	Long-term potentiation
MDD	Major depressive disorder
MEP	Motor evoked potential
ML	Mediolateral
mPFC	Medial prefrontal cortex
MR-ARFI	Magnetic resonance acoustic radiation force imaging
MRgFUS	Magnetic resonance-guided focused ultrasound
MRI	Magnetic resonance imaging
NMDAR	N-methyl-D-aspartate receptor
NTS	Nucleus tractus solitarius
PCC	Posterior cingulate cortex
PD	Pulse duration
PFC	Prefrontal cortex
pp	Perforant path
PRF	Pulse repetition frequency

PS	Population spike
PSD	Power spectral density
PVDF	Polyvinylidene fluoride
PZT	Lead zirconate titanate
RFB	Radiation force balance
RoI	Region of interest
RoC	Radius of curvature
SCC	Subcallosal cingulate cortex
sCT	Synthetic computed tomography
SD	Sonication duration
SDR	Skull density ratio
SSEP	Somatosensory evoked potential
SSRI	Selective serotonin reuptake inhibitor
tDCS	Transcranial direct current stimulation
tFUS	Transcranial focused ultrasound
TMS	Transcranial magnetic stimulation
VS	Ventral striatum

Symbols

a	Attenuation coefficient	[dB/(cm·MHz ⁿ)]
c	Speed of sound	[m/s]
ρ	Mass density	[kg/m ³]
p	Acoustic pressure	[Pa]
$\hat{p}$	Complex pressure amplitude	[Pa]
$\mathbf{v}$	Particle velocity	[m/s]
I	Acoustic intensity	[W/m ²]
P	Acoustic power	[W]
F_{rad}	Acoustic radiation force	[N]
Z	Acoustic impedance	[Rayl]
f	Frequency	[Hz]
ω	Angular frequency	[rad/s]
k	Wavenumber	[1/m]
λ	Wavelength	[m]
θ	Incidence angle	[°]
φ	Phase angle	[rad]
$\mathbf{r}$	Position vector	[m]
t	Time	[s]
D	Aperture diameter	[mm]
F	Geometrical focal length	[mm]
V	Applied voltage	[V]
E	Electric field	[V/m]
D	Electric displacement	[C/m]
S	Strain tensor	—
T	Stress tensor	[Pa]
C^E	Elastic stiffness tensor	[Pa]
e	Piezoelectric stress coefficient	[C/m ²]
ε^S	Dielectric permittivity	[F/m]
ε	Small perturbation parameter	—
$\mathbf{T}$	Momentum-flux tensor	[Pa]

1. Introduction

1.1. Motivation

Brain disorders are increasingly understood not only as consequences of isolated lesions or single molecular abnormalities, but also as disturbances of distributed neural circuits. This shift has motivated growing interest in interventions capable of modulating brain activity at the network level (Khachaturian et al., 2025). Established neuromodulation approaches, including electroconvulsive therapy (ECT) and deep brain stimulation (DBS), have shown clinical efficacy in disorders such as treatment-resistant depression. However, their broader use is constrained by important practical and conceptual limitations. ECT remains affected by stigma and limited spatial specificity, while DBS requires surgical implantation. These methods are therefore not generally suitable for non-invasive experimental perturbation of neural circuits in healthy volunteers.

Non-invasive neuromodulation techniques have addressed some of these limitations. Transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) have provided useful means of modulating brain activity without surgery and have become important tools in neuroscience. Nevertheless, both approaches remain constrained by relatively diffuse field distributions and limited ability to selectively engage deep brain structures.

Transcranial focused ultrasound (tFUS) has emerged as a potentially important addition to this neuromodulation landscape. In principle, tFUS can be delivered non-invasively while producing a more spatially confined acoustic focus at greater depth than conventional non-invasive electrical or magnetic stimulation. These features make it attractive both as a tool for investigating brain function and as a candidate modality for future clinical neuromodulation.

A growing body of work, ranging from cellular preparations to animal models and human studies, suggests that ultrasound can influence neural systems across multiple spatial and functional scales, and these are reviewed in Chapter 2. However, the practical value of tFUS depends not only on whether ultrasound can modulate neural activity, but also on whether the delivered acoustic field can be characterised and interpreted with sufficient confidence. This distinction is central. In transcranial applications, the free-field output of a transducer cannot be assumed to represent the pressure, intensity or focal distribution delivered to the intended neural target. Before reaching the brain, the ultrasound beam must pass through the skull, a dense, heterogeneous and subject-specific structure that can attenuate, refract, distort and redistribute acoustic energy.

Uncertainty in transcranial delivery therefore remains a principal obstacle to the reliable use of tFUS in neuromodulation research. If the delivered field is unknown, then observed physiological or behavioural effects cannot be interpreted confidently in relation to acoustic dose. Conversely, null or inconsistent findings may reflect not only biological variability, but also unrecognised differences in skull transmission, focal distortion or target engagement.

1.2. Aims and structure

This thesis addresses the physical and methodological problems that limit confident interpretation of transcranial focused ultrasound delivery. Its overall aim is to reduce the gap between the conceptual appeal of tFUS and its physically interpretable use in practice. The work focuses primarily on the sub-megahertz frequency range commonly used in tFUS neuromodulation and examines how acoustic source behaviour, skull properties, transducer-skull geometry and modelling assumptions influence estimates of intracranial pressure delivery.

The specific aims of the thesis are to establish an experimental framework for characterising the acoustic sources used throughout the work; to quantify how human

skull specimens modify transmitted ultrasound, including insertion loss, focal distortion, focus shift, focus expansion and spacing-dependent interference; to develop a CT-informed analytical model for rapid estimation of transcranial insertion loss under defined acoustic and geometric assumptions; to benchmark numerical skull-modelling strategies against measured intracranial acoustic fields; and to evaluate, in a preliminary in-vivo setting, whether a physically characterised tFUS system can be integrated with hippocampal electrophysiology while preserving interpretable recordings.

The thesis is organised into seven chapters. Chapter 2 reviews the relevant literature on neuromodulation, focused ultrasound mechanisms, tFUS studies in animals and humans, and the physical problem of skull-mediated ultrasound transmission. Chapter 3 establishes the acoustic source-characterisation framework, linking piezoceramic behaviour to measurable acoustic output and experimental field mapping. Chapter 4 investigates transcranial transmission through ex-vivo human skull specimens, with particular attention to insertion loss, beam distortion, skull morphology, insertion angle and transducer-skull spacing. Chapter 5 develops a CT-informed analytical model for rapid prediction of transcranial insertion loss and benchmarks it against simulation and measurement. Chapter 6 evaluates full-wave skull-modelling strategies for predicting intracranial acoustic fields and presents a preliminary in-vivo feasibility study combining cone-coupled tFUS with hippocampal electrophysiology. Finally, Chapter 7 synthesises the findings and discusses their implications for future tFUS neuromodulation research.

2. Literature review

This chapter establishes the clinical, biological and physical context for the work presented in this thesis. Brain disorders are first considered through the example of major depressive disorder, which is used to illustrate the clinical need for improved neuromodulation approaches. Neuromodulation is then introduced, and the principal stimulation methods used in clinical and research settings are reviewed. Focused ultrasound is subsequently examined as an emerging neuromodulation technique, including its reported neuromodulatory effects and proposed biophysical mechanisms. Finally, the skull is considered as the principal physical barrier to transcranial ultrasound delivery, motivating the need to predict, evaluate and validate the transmitted acoustic field.

2.1. Brain disorders

Disorders of the brain and nervous system are recognised as leading causes of disability worldwide and remain major challenges in modern medicine (Feigin et al., 2019). Whether neurological or psychiatric in presentation, these conditions often involve disruption of the neural circuits that regulate information processing, plasticity and behavioural output. In this sense, many brain disorders are not simply disorders of discrete molecular, cellular or anatomical elements, but disorders of network function.

This systems-level perspective has important implications for treatment. If symptoms arise from abnormal circuit dynamics, effective intervention may require more than correction of a single biochemical pathway. Pharmacological therapy remains essential, particularly where disease expression is driven by molecular and synaptic dysfunction. However, in many disorders, especially those with chronic or treatment-resistant forms, indirect molecular intervention may be insufficient to restore dysfunctional network activity. This has driven increasing interest in therapeutic strategies that act more directly on neural activity, with the aim of modulating excitability, synaptic function and circuit dynamics in a controlled manner.

Among the many disorders in which this problem becomes especially clear, major depressive disorder (MDD) is particularly instructive. MDD is strongly associated with disturbances in synaptic plasticity (Khairova et al., 2009) and large-scale circuit regulation (Kaiser et al., 2015), rather than with a single isolated lesion. It therefore provides a useful framework for understanding why progressively more direct forms of intervention have become an important focus of both basic and translational neuroscience.

2.1.1. Major depressive disorder

MDD is one of the most common psychiatric disorders (World Health Organization, 2017). The prevalence of suicidal attempt in patients with MDD has been reported to be more than seven times higher than in healthy controls (Cai et al., 2021). At the

mechanistic level, one plausible contributor to the depressive pathology is astrocytic dysfunction (Cui et al., 2024). Although astrocytes do not generate action potentials (APs) in the same manner as neurons, they serve essential roles in tripartite synapses, where astrocyte-neuron interactions contribute to the regulation of synaptic transmission and plasticity. The physiological basis of these interactions remains under investigation (For details, see (Cui et al., 2024)), but one proposed pathway involves calcium ion (Ca^{2+}) vesicular exocytosis and gliotransmitter release (Zhou et al., 2019). In normal synaptic physiology, glutamate, the principal excitatory neurotransmitter released by neurons, is taken up by astrocytes and converted into non-excitatory glutamine, thereby contributing to the regulation of synaptic activity (Figure 2-1). When this regulatory function is impaired, disrupted astrocytic glutamate uptake or metabolism may alter extracellular glutamate regulation and contribute to excitotoxic signalling (Zou et al., 2010). Consistent with this view, post-mortem studies of patients with MDD-related symptoms have reported elevated glutamate levels in the frontal cortex (Küçükbrahimoğlu et al., 2009), together with reduced astrocyte number or density in the prefrontal cortex (PFC), hippocampus, and anterior cingulate cortex (ACC) (Wray et al., 2018). In vivo studies further support functional alterations in these frontal and temporal regions in patients with MDD, including reduced synaptic density (Holmes et al., 2019), and either increased (Yoshimura et al., 2014) or decreased (Grimm et al., 2009) activity in response to different stimuli compared with healthy controls.

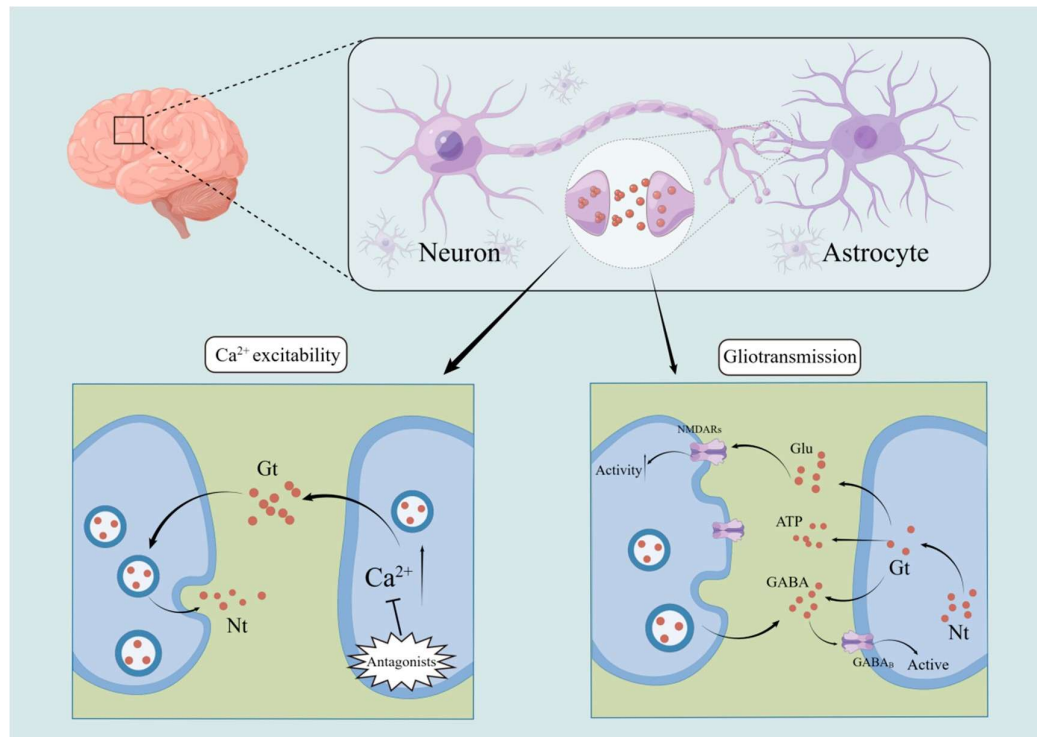


Figure 2-1 Schematic illustration of astrocyte–neuron interactions relevant to depressive pathology. Adapted from (Yao et al., 2023). The schematic illustrates the bidirectional signalling between astrocytes and neurons. Neuronal activity can activate astrocytes, while activated astrocytes, particularly through increased intracellular Ca^{2+} , can in turn regulate synaptic transmission by releasing gliotransmitters (Gt) such as glutamate (Glu) and ATP. These signals act on neuronal receptors, including ionotropic glutamate receptors such as NMDARs, thereby altering postsynaptic excitability and synaptic transmission. In addition, astrocytes can be activated by GABAergic interneurons through GABA_B receptors, illustrating their integration into both excitatory and inhibitory circuits. Astrocyte-to-neuron signalling can be blocked by ionotropic glutamate receptor antagonists.

2.1.2. Current treatment approaches

Pharmacological solution

Given the evidence that depression is associated with disturbed synaptic plasticity and astrocyte-mediated glutamatergic regulation, pharmacological treatment remains an important first-line approach. One notable example is ketamine, whose antidepressant effects are thought to involve brain-derived neurotrophic factor (BDNF), a protein released by both neurons and astrocytes that plays an important role in neuronal development, synaptic plasticity, and neurotransmission (Bathina & Das, 2015). Reduced BDNF signalling has been associated with depressive pathology, and ketamine is thought to exert part of its antidepressant effect through glutamatergic modulation

and downstream enhancement of BDNF-related plasticity, including increased BDNF expression in the PFC (Quesseveur et al., 2013) and hippocampus (Zanos et al., 2016).

Although ketamine can alleviate MDD-related symptoms within days, in contrast to the slower effects of conventional antidepressants such as selective serotonin reuptake inhibitors (SSRIs), its use remains limited by incomplete efficacy and acute side effects. Reported adverse events include psychiatric symptoms such as anxiety and irritability, as well as psychotomimetic effects such as dissociation and perceptual disturbance, which have been reported in approximately 40% and 70% of cases, respectively (Short et al., 2018). Moreover, approximately one in three patients reportedly fails to achieve remission (Voineskos et al., 2020). By general consensus, patients who do not respond to two adequate antidepressant trials may be classified as having treatment-resistant depression (TRD). Under a more stringent criterion requiring symptomatic remission, as many as 55% of patients with MDD may meet the criteria for TRD (McIntyre et al., 2023).

Patients with TRD typically experienced more severe depressive symptom and have a yearly suicide-attempt rate of nearly 5% (Bergfeld et al., 2018). For this reason, additional approaches, including psychotherapy and somatic treatments, are often considered either alone or in combination, although their therapeutic effects may emerge over different timescales.

Electroconvulsive therapy

The current ‘last resort’ for effectively treating TRD is electroconvulsive therapy (ECT), which acts not by directly correcting astrocytic biochemistry but by inducing circuit-level reorganisation. ECT administers brief alternating pulses through scalp electrodes, the induced in-situ electric-field (E-field) strength has been empirically estimated at 0.7 to 2.5 V/cm, approximately two- to seven-fold higher than the threshold for neuronal activation (Deng et al., 2011; W. H. Lee et al., 2016). This acute surge of electrical activity overwhelms brain’s normal communication signals between nerve cells, causing a generalized seizure that transiently disrupts large-scale functional connectivity. The subsequent physiological cascades are thought to promote neuroplasticity and circuit rewiring (Ousdal et al., 2022).

Clinically, ECT demonstrates high efficacy in reducing depressive severity compared with pharmacological interventions, including ketamine (Rhee et al., 2022), with a reported remission rate of 75% in large-scale trial (Husain et al., 2004). However, adverse effects such as cognitive impairment may persist for weeks after treatment (Semkowska & McLoughlin, 2010). One study suggests that these cognitive effects may arise from the widespread of E-field, because its non-focal nature can engage broad brain regions (Deng et al., 2023), while another shows that cognitive burden increases with greater applied E-field strength (Abbott et al., 2024). Although individualised stimulation parameters may help mitigate these adverse effects without compromising efficacy, their precision is limited by the challenge of accurately estimating subject-specific skull electrical conductivity, which is anisotropic and heterogeneous across individuals (Deng et al., 2011). Together with the entrenched stigma surrounding ‘shock therapy’ and the concerns about memory loss, these limitations may contribute to reluctance among some patients to undergo the procedure (O’Reardon et al., 2006).

2.2. Neuromodulation

Given the limitations in acceptability and tolerability associated with pharmacological treatments and ECT for disorders such as MDD and TRD, there is a clear need for alternative approaches that can offer therapeutic benefits without the same burden of adverse effects or stigma.

Neuromodulation, in this context, is broadly defined as the modulation of nervous-system activity through targeted interaction with neural tissue, typically with the aim of producing adjustable and non-lesional effects (Krames et al., 2018). Although the term can, in a broad sense, encompass pharmacological agents that alter neuromodulatory tone, the focus here is on neuromodulation produced by physical energy delivery. These methods aim to influence the excitability and connectivity of defined brain regions or pathways, thereby altering pathological network dynamics in a manner that is, in principle, controllable and reversible. Both invasive approaches,

involving implanted devices, and non-invasive approaches, applied from outside the skull, fall under this definition.

The following subsections review major neuromodulation modalities according to the primary physical mechanism by which energy is delivered to the nervous system. This organisation provides a basis for comparing their respective strengths and limitations, particularly in terms of invasiveness, spatial precision, stimulation depth, field predictability and clinical maturity.

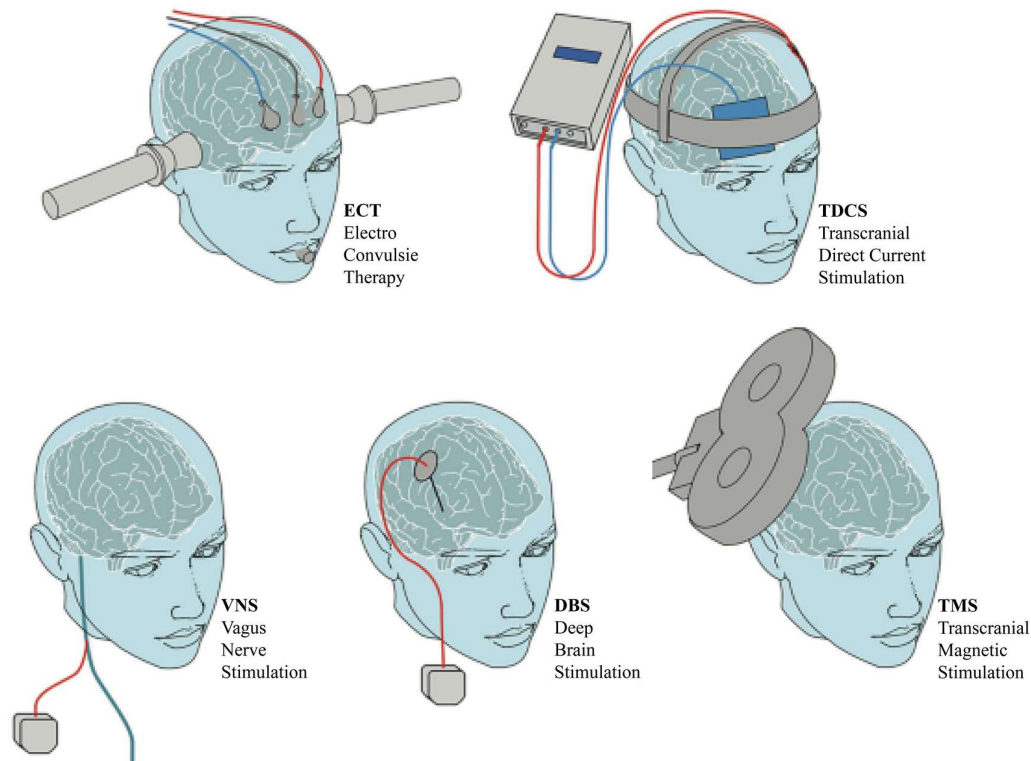


Figure 2-2 Comparative overview of major neuromodulation modalities discussed in this thesis, including ECT, transcranial direct current stimulation (tDCS), vagal nerve stimulation (VNS), deep brain stimulation (DBS), and transcranial magnetic stimulation (TMS). Adapted from (Arns & Fitzgerald, 2024).

2.2.1. Electrical modalities

Biophysical mechanisms

Electrical activity has been known to exist in nerves since the 19th century, and this activity is now understood to take the form of transient changes in membrane potential known as APs, which the nervous system uses to transmit information, for example in

the control of muscle contraction (Enoka et al., 2019). The biophysical basis of AP was first characterised in detail by Hodgkin and Huxley in their studies of the squid giant axon. By combining intracellular recordings with the voltage-clamp method, they were able to hold the membrane potential at defined levels and measure the resulting transmembrane currents. This approach allowed the subsequent identification of voltage-gated ion channels conductance and their activation threshold (Hodgkin & Huxley, 1952).

In human neurons, the resting membrane potential is approximately -70 mV, when a depolarising input brings the membrane potential to approximately -55 mV, voltage-gated sodium ion (Na^+) channels open; and Na^+ , which are more concentrated outside the cell, flow into the neuron. This inward current drives the membrane potential towards positive values, with the peak of the AP often reaching to above +30 mV. As the membrane depolarises, Na^+ channels become inactivated and potassium ion (K^+) channels open. K^+ , which are more concentrated inside the cell, then flow out, returning the membrane potential towards its resting value. Subsequent leak conductance and Na^+ / K^+ -ATPase restore the ionic gradients that support the resting potential.

Once initiated, the AP propagates along the axon to the presynaptic terminals. The depolarization at the terminal opens voltage-gated Ca^{2+} channels, and resulting influx of Ca^{2+} triggers the release of neurotransmitter into synaptic cleft. The released neurotransmitter binds to receptors on the postsynaptic membrane at the dendrites of the connected neuron. Those ionotropic receptors signalling the increased permeability to Na^+ and Ca^{2+} , thereby depolarizing the postsynaptic membrane and then triggering AP initiation in the postsynaptic cell. Repeated activation of such synapses can induce longer-lasting changes in synaptic efficacy, for example, enhancement of N-methyl-D-aspartate receptor (NMDAR) function, which strongly regulate Ca^{2+} entry into the postsynaptic neuron, facilitates long-term potentiation (LTP) like increase in synaptic strength, whereas reduced NMDARs activity can favour depression-like changes (Monte-Silva et al., 2013).

External electrical stimulation acts on this substrate by generating spatial gradients in extracellular potential. These gradients induce local changes in transmembrane voltage

along neuronal processes and can bias the probability of AP generation (A. Liu et al., 2018).

Transcranial direct current stimulation

The clinical success of ECT demonstrates that perturbing the functional integrity of widespread neural networks can alleviate depressive symptoms. A natural question is therefore whether reducing the intensity of stimulation, while maintaining a similar scalp electrode configuration, can still confer antidepressant effects with a more favourable tolerability profile. Transcranial direct current stimulation (tDCS) adopts a closely related architecture to ECT, but unlike ECT, tDCS does not aim to induce seizure activity, but to bias cortical excitability by applying a current that is several orders of magnitude lower in amplitude over considerably longer periods, typically 20 to 30 minutes per session over several weeks. Clinical studies report remission rates on the order of 40% to 50%, with adverse effects generally limited to mild and transient sensations such as tingling at the electrode sites. (Woodham et al., 2025).

Despite this favourable tolerability profile, tDCS is characterised by substantial inter-subject variability in outcomes, which has limited its reliability as a stand-alone treatment. The generated electric fields are weak and still spatially diffuse, extending across broad cortical and subcortical regions rather than being confined to a single target. As a result, stimulation interacts with each individual's neuroanatomy and baseline network state in a complex and only partially controllable manner. Differences in skull morphology and composition further distort current distribution, such that nominally identical scalp current can produce markedly different intracranial field strengths between subjects (Benwell et al., 2015). Together with uncertainty in the dose-response relationship at low field magnitudes, these factors make it difficult to predict or standardise the cognitive and affective effects of tDCS across patients.

Vagal nerve stimulation

The limited spatial specificity of conventional non-invasive stimulation has motivated increasing interest in approaches that can more selectively engage defined neuronal pathways. Pharmacological evidence provides one route for identifying circuits relevant to mood regulation. The antidepressant action of SSRIs, although increasingly debated and clearly insufficient to explain the full pathophysiology of depression

(Moncrieff et al., 2022), implicates serotonergic signalling in mood regulation (Boschloo et al., 2023). The dorsal raphe nucleus (DRN), located in the midbrain, is the principal source of ascending serotonergic projections to prefrontal, limbic and subcortical regions (McDevitt et al., 2014). Ketamine has also been reported to interact with noradrenergic (a crucial hormone) signalling (Liebe et al., 2018). Noradrenaline in the forebrain is largely synthesized by specialized neurons in the locus coeruleus (LC), a pontine nucleus with widespread projections to cortex and hippocampus and exerts a strong modulatory influence on affective state (Bari et al., 2019). Thus, these observations identify the DRN and LC as important nodes in mood-regulating networks.

Both the DRN and LC receive dense afferent input from the nucleus tractus solitarius (NTS), a major termination site for visceral signals carried by afferent vagal fibres. This anatomical arrangement provides a route by which peripheral stimulation of the vagus nerve could influence central monoaminergic system. On this basis, vagal nerve stimulation (VNS) has been proposed as a means of modulating mood by indirectly engaging DRN and LC activity (Carreno & Frazer, 2017).

Clinically, VNS is implemented by surgically implanting an electrode around the left cervical vagus nerve, a peripheral target that is more surgically accessible than intracranial brainstem nuclei. Stimulation is delivered in short trains of pulses with amplitude in the range comparable to or lower than those used in tDCS but confined to the nerve rather than flowing across the scalp-skull-brain interface. In TRD, preliminary studies indicate that approximately half of patients exhibit a sustained clinical response over months to years of chronic VNS, although remission rates remain modest and the evidence base is still evolving (Carreno & Frazer, 2017).

Experimental work provides mechanistic support for engagement of monoaminergic nuclei. In animal models, chronic VNS increases expression of activity-dependent markers such as c-Fos in both LC and DRN (Cunningham et al., 2007). These markers are also elevated in structures including thalamus, cingulate cortex, frontal cortex and hippocampus (Carreno & Frazer, 2017), indicating that VNS effects are not confined to a single circuit but propagate through broader interconnected networks. Consistent with immunohistochemical findings, electrophysiological recordings demonstrate altered

activity of noradrenergic and serotonergic neurons during stimulation (Manta et al., 2009).

Deep brain stimulation

VNS represents a step towards more pathway-oriented neuromodulation by exploiting a defined peripheral afferent route to brainstem nuclei implicated in mood regulation. However, because its effects remain distributed across multiple interconnected regions, it is difficult to dissociate the contribution of individual nodes from that of the wider network. Achieving greater anatomical specificity requires techniques that can deliver stimulation directly to circumscribed brain structures, deep brain stimulation (DBS) adopts this strategy by surgically implanting stimulating leads into predefined deep targets.

In DBS, one or more leads, typically with a diameter of approximately 1 mm are inserted unilaterally or bilaterally through burr holes in the skull. Magnetic resonance imaging (MRI) guidance is commonly used to plan and verify electrode placement. Stimulation is delivered as short pulses, and parameters can be titrated according to individual response and tolerability. Several targets have been explored in TRD, including the subcallosal cingulate cortex (SCC) and ventral striatum (VS) (Malone et al., 2009), to name but a few. Although these regions differ in anatomical location and connectivity, they are embedded within mood-regulating networks that overlap with those influenced indirectly by VNS.

Not surprisingly, DBS has shown promising outcomes in TRD. Collective data suggest that more than half of the treated patients achieved meaningful clinical response, and approximately one third reach remission, with some series reporting superior outcomes compared with VNS (Johnson et al., 2024). Refinement of targeting strategies continues, with the aim of positioning electrodes more precisely in relation to network hubs associated with optimal response.

Electrophysiological studies further suggest that DBS can produce measurable signatures of target engagement. EEG recordings have revealed stimulation-induced changes in oscillatory activity, including increased theta and gamma power in prefrontal cortex. Local field potential (LFP) recordings showed modulation of beta activity at the

stimulation site, raising the possibility that specific oscillatory features could serve as biomarkers of target engagement (Smart et al., 2018).

Notwithstanding, these advantages come at the cost of substantial invasiveness and restricted accessibility. While DBS is well established for movement disorders such as Parkinson's disease and essential tremor, its application in TRD remains investigational. The requirement for intracranial surgery is a major barrier to patient and clinician acceptance. Approximately 90% of screened TRD candidates did not ultimately proceed to implantation, and more than one third of participant withdrew (Ramasubbu et al., 2021), reflecting concerns about surgical risk and uncertain benefit. These recruitment challenges have limited translation from research settings into clinical practice for MDD.

2.2.2. Magnetic stimulation

The electrical techniques discussed above either deliver current directly through scalp electrodes, as in ECT and tDCS, or require implanted hardware to stimulate peripheral nerves or deep brain targets, as in VNS and DBS. A further question is whether an electric field can be generated within the brain non-invasively, without passing current directly through the scalp and skull or implanting electrodes. Transcranial magnetic stimulation (TMS) was developed in the mid-1980s as an answer to this question, using Faraday's law of electromagnetic induction to induce electric currents in cortical tissue with time-varying magnetic fields (Barker et al., 1985).

In TMS, a brief, high-intensity current pulse passes through a coil placed on the scalp. This pulse generates a rapidly changing magnetic field, typically on the order of 1 to 3 Tesla at the coil surface. The magnetic field is only weakly attenuated by the scalp and skull compared with currents delivered directly through scalp electrodes. According to Faraday's law, the rapid change in magnetic flux induces an electric field in the underlying conductive brain tissue. The spatial distribution of the induced field depends on coil geometry and position. The widely used figure-of-eight coil design produces a relatively focal region of maximal field strength beneath the intersection of the two loops, with effective stimulation depths typically in the range of 2 to 4 cm (Siebner et al., 2022).

The induced field drives current flow in a manner analogous to electrical stimulation. When sufficiently strong, it can depolarise neurons to threshold and elicit APs. At the cellular level, the elements most likely to be recruited are axons, particularly those oriented approximately parallel to the induced E-field and tangential to the cortical surface, as their activation thresholds are lower than those of somata and dendrites. The most direct evidence for AP generation comes from stimulation of primary motor cortex, where single TMS pulses evoke motor evoked potentials in contralateral limb muscles and can produce visible muscle twitches. TMS can also induce longer-lasting changes in cortical excitability that resemble LTP-like plasticity (Klomjai et al., 2015).

Clinically, TMS is applied non-invasively with the coil placed over the cortical target. In MDD, stimulation is typically directed to the prefrontal cortex. Meta-analyses support a significant antidepressant effect, with response and remission rates reaching approximately 60% to 80 % and 30 % to 60 %, respectively (Sackeim et al., 2020). TMS also has a favourable safety profile, which has long been cleared by the U.S. Food and Drug Administration (FDA) for the treatment of MDD.

Nevertheless, TMS also has inherent limitations. The decay of the induced electric field with depth restricts effective stimulation mainly to superficial cortical regions, making it difficult to engage deep structures directly compared with DBS. Although figure-of-eight coils provide improved spatial precision compared with older circular coils, the induced fields still extend over centimetre-scale regions of cortex, so the volume of tissue influenced by a given stimulation site remains relatively large. This spatial spread limits the ability to isolate the contributions of specific pathways and complicates mechanistic interpretation of clinical and behavioural effects. Finally, the therapeutic efficacy of TMS depends sensitively on stimulation parameters such as frequency, intensity, pulse pattern and total dose (Morriss et al., 2024). Despite extensive empirical work, the optimal combinations of these parameters for different patient groups and symptom profiles remain under active investigation.

2.3. Focused ultrasound neuromodulation

Focused ultrasound (FUS) has emerged as a candidate neuromodulation modality because it addresses several limitations of the methods reviewed in the preceding section. Unlike tDCS and TMS, it has the potential to deliver acoustic energy to deeper targets with a more restricted focal volume; unlike DBS, it does not require implanted intracranial hardware.

This feature is particularly relevant for the brain, where many clinically and scientifically important targets are located below the cortical surface. Structures such as the thalamus, hippocampus, amygdala and substantia nigra are difficult to access selectively using conventional non-invasive stimulation methods (Glasser et al., 2016). At appropriate frequencies, ultrasound can be focused into a restricted acoustic volume, commonly described using focal pressure or intensity contours. (Naftchi-Ardebili et al., 2024). This potential combination of non-invasive access, depth reach and spatial selectivity has made tFUS attractive for both therapeutic neuromodulation and systems-level interrogation of brain circuits.

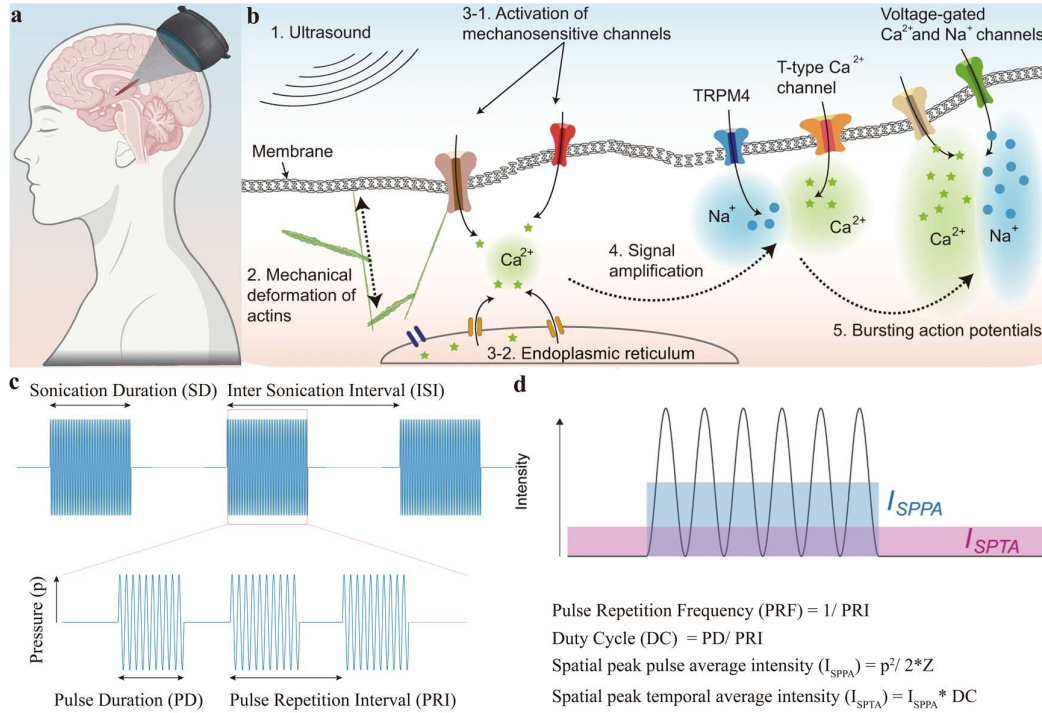


Figure 2-3 Overview of focused ultrasound neuromodulation mechanisms and stimulation parameters (a) Schematic illustration of tFUS neuromodulation, adapted from (Arulpragasam et al., 2026). (b) Proposed molecular pathway for ultrasound-induced neuronal excitation. Ultrasound is suggested to initiate Ca^{2+} entry through mechanosensitive ion channels, particularly TRPC1 and TRPP2, producing an initial rise in intracellular Ca^{2+} . This response may be further supported by intracellular Ca^{2+} release from the endoplasmic reticulum. The Ca^{2+} signal is then amplified by downstream channels, including TRPM4, which promotes inward Na^+ current, together with voltage-gated Ca^{2+} channels such as T-type channels. The combined effect is stronger membrane depolarisation and the generation of bursting APs. Adapted from (S. Yoo et al., 2022a). (c) Representation of the principal temporal and acoustic parameters used in transcranial ultrasound stimulation, including pulse duration (PD), pulse repetition frequency (PRF), duty cycle (DC), sonication duration (SD), and inter-sonication interval (ISI). (d) Representation of the acoustic intensities relationship. Z represents acoustic impedance. Adapted from (Murphy et al., 2025).

2.3.1. Biophysical mechanisms

Despite growing experimental evidence, the molecular and biophysical mechanisms of ultrasound neuromodulation remain incompletely resolved. Early evidence that ultrasound can influence nervous tissue dates back to the 1950s, when Fry and colleagues reported that high-intensity ultrasound temporarily suppressed activity in the crayfish ventral nerve cord and the frog spinal cord (W. J. Fry et al., 1950). Several decades later, experiments in mammalian brain showed that hippocampal neurons *in vitro* could be both suppressed and excited by ultrasound (Bachtold et al., 1998).

However, these early studies typically used exposure levels at which heating, cavitation or tissue disruption could not be excluded.

A major shift occurred when low-intensity, low-frequency ultrasound was shown to modulate neuronal function without overt tissue damage. Tyler and colleagues showed that brief sonication at low intensities could induce transient changes in intracellular Na^+ and Ca^{2+} levels, detected using fluorescent ion indicators, and could trigger vesicle exocytosis at synaptic terminals, thereby altering synaptic transmission (Tyler et al., 2008). One proposed mechanism involves mechanosensitive ion channels that respond to changes in membrane tension. Cells maintain a stable internal osmotic pressure relative to their environment and are therefore at risk of lysis if the membrane cannot accommodate volume changes (Kung, 2005). To mitigate this, mechanosensitive channels in the membrane act as ‘emergency valves’ that open when lateral tension in the lipid bilayer increases. Structural and functional studies indicate that as membrane tension rises, the bilayer thins and the ‘gatekeeper’ lipids that occlude the channel pore are displaced. This conformational change can place the channel in a subconducting or partially open state, allowing ions to pass through (Y. Zhang, Daday, et al., 2021). Ultrasound, by exerting oscillatory mechanical forces on the membrane and the surrounding medium, can transiently increase membrane tension and thereby promote opening of such mechanosensitive channels.

Recent cellular work has begun to identify candidate molecular pathways. Yoo and colleagues used 300 kHz pulsed ultrasound with a 500 ms exposure at an intensity of approximately $15\text{W}/\text{cm}^2$, and reported Ca^{2+} influx across the plasma membrane. Pharmacological manipulations indicated that this entry occurs primarily through mechanosensitive channels, including TRPC1 and TRPP2. The resultant increase in intracellular Ca^{2+} concentration activates the non-selective cation channel TRPM4, which further contributes to Na^+ influx (S. Yoo et al., 2022a). Although the precise contribution of each channel type and the detailed biophysical coupling between the acoustic field and the membrane remain under active investigation, these data support a model in which ultrasound modulates neuronal excitability by engaging mechanosensitive ion channels via changes in membrane tension, in contrast to electrical stimulation, which directly alters transmembrane voltage. However, the

relationship between acoustic exposure, cellular response and circuit-level outcome remains incompletely defined.

2.3.2. Early translational studies

Against this background, transcranial focused ultrasound (tFUS) stimulation has begun to be explored in mood-related circuits that are relevant to the clinical motivation introduced earlier. One early study targeted the amygdala in individuals with mood, anxiety, and trauma-related symptoms and reported reduced amygdala activation after stimulation, accompanied by decreased activity in connected regions such as the hippocampus. Approximately half of the participants were reported to meet response criteria on standardised symptom scales (Barksdale et al., 2025). Another study targeting SCC reported increased functional connectivity within prefrontal circuits and reduced connectivity with hippocampal regions. In this cohort of patients with TRD, 61% were reported to achieve a clinically meaningful reduction in depressive symptoms and 40% reached remission (Attali et al., 2025). In both studies, stimulation was reported to be well tolerated, with no serious adverse events and only mild, transient side effects.

These findings are encouraging, but they should not be interpreted as establishing tFUS as an effective treatment for mood disorders. Existing studies remain small and exploratory, and the acoustic exposure regimes differ across protocols. The extent to which carrier frequency (f), pulse duration (PD), pulse repetition frequency (PRF), duty cycle (DC), sonication duration (SD) and total acoustic dose influence efficacy and safety remains insufficiently defined, and systematic dose–response studies in larger populations are still needed to define optimal operating procedures. In addition, individual differences in skull anatomy and acoustic properties introduce variability in the intracranial field, complicating both interpretation and replication.

2.3.3. Cortical neuromodulation studies

Beyond the early translational studies described above, tFUS has been investigated more broadly as a tool for functional neuromodulation in both cortical and subcortical brain regions. Across this literature, a wide variety of experimental approaches have been used to assess its effects, including electrophysiological recordings, behavioural

paradigms, and functional imaging. The reported effects are diverse, but a recurring pattern is that stimulation outcome depends strongly on waveform and state rather than anatomical target alone. Full ultrasound exposure parameters for representative studies are summarised in APPENDIX A.

Somatosensory cortex

Somatosensory cortex studies provide some of the clearest evidence that tFUS can alter sensory processing. In mouse primary somatosensory cortex (S1), pulsed stimulation at 510 kHz with short pulses and sub-second-to-second exposure was associated with earlier sensory-evoked responses and altered Ca^{2+} activity patterns (Fisher & Gumenchuk, 2018). Extending these physiological observations to behaviour in freely moving animals, one study employed a miniature head-mounted transducer targeting the mouse S1 barrel field produced changes in local spiking activity and lateralised head-turning responses, linking S1-targeted stimulation to quantifiable behavioural output (G. Li et al., 2019). In non-human primates, MRI-guided tFUS targeting the functionally defined S1 hand representation suppressed tactile-evoked blood-oxygen level-dependent (BOLD) responses at the target while also modulating downstream interconnected regions, suggesting that even focal stimulation can influence distributed somatosensory networks (P. F. Yang et al., 2021).

Human studies provide complementary evidence under controlled experimental conditions. In left S1, a commonly cited 500 kHz protocol ($f = 500$ kHz, PD = 0.36 ms, PRF = 1 kHz, $I_{\text{sppa}} = 23.87$ W/cm², $p = 800$ kPa, SD = 0.5 s) attenuated median-nerve somatosensory evoked potentials, altered sensory-evoked oscillatory power and improved tactile discrimination relative to sham stimulation (Legon et al., 2014). Analyses using the same core waveform further suggested that tFUS reshaped intrinsic and evoked phase dynamics, particularly in the beta band, supporting modulation of local oscillatory state rather than a purely non-specific arousal effect (Mueller et al., 2014). Extending beyond single-site S1 targeting, dual-transducer stimulation of S1 and S2 transiently evoked contralateral tactile percept, demonstrating the feasibility of perturbing multiple somatosensory nodes in humans (W. Lee, Chung, et al., 2016). More recent task-based work combining multichannel EEG with electrophysiological source imaging reported that finger-region S1 stimulation increased vibration-

discrimination accuracy and enhanced both sensor- and source-domain somatosensory responses (C. Liu et al., 2021), broadly consistent with earlier behavioural improvements under S1 stimulation (Legon et al., 2014). Nevertheless, the direction of effect should not be assumed to be uniformly facilitatory. Subsequent findings suggest that behavioural changes may also reflect inhibitory modulation under certain PRF regimes, highlighting the dependence of perceptual outcomes on stimulation settings and physiological state (Legon, Bansal, et al., 2018).

Motor cortex

Motor cortex studies illustrate particularly clearly that tFUS effects are parameter- and state-dependent. In anaesthetised rodents, successful motor-cortex stimulation emerged within constrained pulsing regimes, with pulsed sonication reducing threshold relative to continuous exposure (Kim et al., 2014). Further work in awake behaving rodents elicited movements across several body regions and showed that awake animals had higher effective thresholds but shorter response latencies than anaesthetised animals, while inter-animal variability in response rate decreased in the awake condition (W. Lee et al., 2018). In awake sheep, stimulation of M1 and its thalamic projection region elicited selective contralateral hindlimb electromyography (EMG) responses, with shorter PDs yielding larger amplitudes (H. C. Kim et al., 2022). Physiological readouts further support state dependence: EMG and LFP responses were followed by cortical blood flow (CBF) changes that scaled monotonically with intensity and SD, whereas deep anaesthesia abolished responses (Yuan et al., 2020).

Human studies broadly preserve this parameter dependence, but the most internally consistent results emerge when they are grouped by waveform family. Short 500 kHz single-train protocols generally point toward brief online suppression. For example, sonication with PRF around 1 kHz and sub-second exposure reduced motor-evoked potential (MEP) amplitude and intracortical facilitation (Legon, Bansal, et al., 2018). Systematic testing within this carrier-frequency family showed that suppression became more reliable with longer SD and lower DC, and was more robust in blocked than interleaved designs (Fomenko et al., 2020). Xia et al. further clarified that this suppressive effect is temporally narrow, occurring around the end of sonication and not reliably transferring to contralateral M1 at rest, which helps explain why durable

aftereffects are often absent with this protocol class (Xia et al., 2021). Likewise, response-inhibition study using a longer 500 kHz M1 exposure also reported sustained MEP suppression (Nakajima et al., 2022), while a parallel fMRI study using a related waveform found spatially confined increases in activation volume without clear BOLD-amplitude changes, suggesting that suppressive electrophysiological regimes may still produce measurable local modulation (Ai et al., 2018).

By contrast, patterned or repetitive 500 kHz protocols more often yield facilitatory or plasticity-like aftereffects. Theta-burst stimulation delivered as an 80 s train increased MEP amplitude for at least 30 min and was accompanied by reduced intracortical inhibition (Zeng et al., 2022). A follow-up study using the same general parameter set reported sustained MEP increases together with altered alpha/beta activity and motor-network connectivity measured with MEG (Sarica et al., 2022). Later pharmacological manipulation did not fundamentally overturn this interpretation, but showed that the facilitatory aftereffect was diminished when cortical excitability was shifted, supporting the view that FUS-induced plasticity depends on the prevailing excitatory–inhibitory balance rather than on a trivially stimulus-locked effect (Shamli Oghli et al., 2023). Longer repetitive stimulation also supports this pattern: repeated M1 stimulation over 15 min increased ipsilateral excitability and transiently shifted interhemispheric balance (Ren et al., 2023; Y. Zhang, Ren, et al., 2021). Similarly, MRI-guided testing showed that a high-PRF/DC pattern increased excitability, whereas a low-PRF/DC pattern decreased it (T. Zhang et al., 2023). A separate 800 kHz protocol reported improved hand tapping, shorter MEP latency and increased MEP amplitude, again indicating that facilitatory motor outcomes can occur under different exposure regimes (M. F. Zhang et al., 2022).

Notwithstanding, not all motor studies show clear modulation. Left M1 stimulation during tonic contraction produced neither concurrent EMG modulation nor changes in excitability (Heimbuch et al., 2022), whereas stimulation of leg motor cortex during voluntary foot movement enhanced movement-related cortical potentials (Yu, Liu, et al., 2021). Diagnostic imaging ultrasound over M1 produced transient MEP facilitation after 2 min of stimulation, supporting the possibility of excitability enhancement under

an alternative exposure regime, although this is not directly comparable to low-frequency tFUS protocols (Gibson et al., 2018).

Visual cortex

Visual modulation remains comparatively under-investigated relative to somatosensory and motor targets, but existing studies reinforce the importance of exposure regime and network context. In rodent primary visual cortex (V1), 690 kHz stimulation with moderate intensity and low PRF transiently suppressed light-evoked responses, whereas increased intensity or PRF shifted the net effect towards modest elevation (Kim et al., 2015). In primate studies, stimulation of the frontal eye field (FEF) during antisaccade task produced measurable behavioural slowing (Deffieux et al., 2013). Follow-up electrophysiology in the supplementary eye field, a reciprocally connected region, revealed heterogeneous single-neuron effects with a roughly balanced distribution of increases and decreases in firing (Wattiez et al., 2017), albeit the causal projections between the two regions been less straightforwardly determine the on-site modulatory effect. More recent work demonstrated remote consequences of FEF stimulation more directly: tFUS to FEF reduced spiking rate in extrastriate visual cortex V4 under one parameter set, whereas neuronal activity increased when PRF was doubled and pulse duration was halved (Yu et al., 2024).

Human visual studies have developed in parallel. Using parameters related to earlier human S1 work (W. Lee et al., 2015a), V1 stimulation was associated with stimulus-locked EEG responses and diffuse visual percepts in a subset of participants, although inter-subject variability did not map straightforwardly onto targeting error or estimated in-situ intensity (W. Lee, Kim, et al., 2016). A comparable responder rate has been reported using diagnostic ultrasound, although the percept was described as spatially and temporally extended, again making this exposure regime difficult to compare directly with low-frequency tFUS (Schimek et al., 2020). More recent systems-level work has shifted from eliciting percept toward testing whether tFUS can bias visual processing in task-relevant directions, in a visual-evoked-potential brain-computer interface paradigm, stimulation of motion-sensitive visual cortex improved online spelling performance relative to multiple control conditions under comparatively low derated exposures (Kosnoff et al., 2024).

Auditory cortex

Evidence for modulation of auditory processing has been reported in animal models using evoked-potential readouts. In deeply anaesthetised rats, sonication suppressed auditory evoked potential (AEP) amplitudes, and a comparable suppression profile was observed in swine under the same stimulation design (Daniels et al., 2018). However, later human work introduced an important interpretive complication. Commonly used tFUS waveforms can produce reliable AEPs through bone-conducted sound, and this auditory signature can be substantially reduced when an appropriate masking tone is delivered via earphones (Braun et al., 2020).

This introduces a non-trivial ambiguity for studies targeting sensory cortices, apparent modulation may partly reflect auditory salience, startle-like processes or incomplete sham matching unless the audible component is adequately controlled. Converging with this concern, parameter refinements such as pulse-envelope ramping and explicit acoustic masking have been reported to reduce subjective audibility (Johnstone et al., 2021).

Higher-order areas

Beyond primary sensory and motor regions, tFUS has also been applied to higher-order cortical areas involved in affective, cognitive and network-level regulation. Early affective work using an 8 MHz imaging probe over posterior frontal cortex reported improved self-reported mood in chronic pain participants, although this exposure regime differs substantially from most low-frequency tFUS neuromodulation protocols (Hameroff et al., 2013). Subsequent PRF studies, often centred on 500 kHz stimulation with low PRF values and sonication durations from tens of seconds to minutes, reported improved global affect, reduced anxiety and altered resting-state connectivity (Reznik et al., 2020; Sanguinetti et al., 2020a). Later study reported decreased midfrontal theta activity together with increased approach and reduced withdrawal behaviour, suggesting that this waveform class can influence affect-regulatory prefrontal systems in large (Ziebell et al., 2023). Stimulation of the right inferior frontal gyrus using a related 500 kHz platform modified the relationship between stimulus probability and cortical alpha activity without simply changing subjective control judgements (Forster et al., 2023).

Clinical and association-cortex studies further extend this evidence base. Repetitive left dorsolateral PFC stimulation over three weeks reduced negative symptoms and improved continuous-performance scores in schizophrenia, suggesting that tFUS effects are not confined to healthy affective paradigms (Zhai et al., 2023). Offline theta-burst stimulation of the posterior cingulate cortex (PCC) or dorsal anterior cingulate cortex (dACC) increased functional connectivity in their respective default-mode and salience networks; PCC stimulation additionally reduced local GABA, indicating that effects can persist for tens of minutes and may differ across nearby association-cortex targets (Yaakub et al., 2023). Finally, bilateral medial PFC stimulation at lower frequency produced DC-dependent EEG redistribution during and after sonication, further supporting parameter-dependent modulation of higher-order cortical networks (Y. G. Kim et al., 2022).

2.3.4. Subcortical neuromodulation studies

Beyond the cerebral cortex, tFUS has also been applied to subcortical structures. This extension is central to the appeal of tFUS, because deep targets such as the thalamus, hippocampus, amygdala and brainstem contribute to arousal, memory, affective regulation, sensory integration and disease-relevant circuit function. The subcortical literature therefore provides an important test of whether tFUS can influence neural systems that are difficult to access selectively with conventional non-invasive stimulation methods.

Thalamic region

Among deep neural targets, the thalamus has received the greatest attention. Early animal work showed that thalamic sonication can alter global brain state: pulsed stimulation around 650 kHz in anaesthetised rats shortened emergence from anaesthesia (S. S. Yoo et al., 2011), whereas lower-intensity stimulation in an epileptic rat model reduced epileptiform bursts and seizure severity (Min et al., 2011). More detailed parametric work then showed that seizure suppression scaled mainly with exposure level and SD, emphasising that dose and temporal exposure are central to interpretation (S. G. Chen et al., 2020). Large-brain studies further indicated that thalamic modulation can be spatially selective: in swine, sensory-thalamic tFUS reversibly suppressed somatosensory evoked potentials (SSEPs) without detectable tissue injury (Dallapiazza

et al., 2017). By contrast, rat ventral posterolateral (VPL) stimulation under a higher-frequency regime produced suppression that tracked focal temperature closely enough to support a predominantly thermal mechanism in that preparation (Darrow et al., 2019).

Human thalamic studies broadly support target relevance while preserving interpretive complexity. Central-thalamic stimulation in patients with chronic disorders of consciousness was associated with behavioural improvement in two of three individuals (Cain et al., 2021), right anterior-thalamic stimulation reduced thermal pain sensitivity (Badran et al., 2020), and S1/VPL targeting in healthy generated FUS-evoked EEG responses and increased thalamic-network functional connectivity, with shorter pulses producing the clearest effects (H. C. Kim et al., 2023). A recent study targeting the lateral geniculate nucleus (LGN), a thalamic relay involved in visual processing, illustrates the increasing spatial precision of phased-array tFUS approaches. Using a helmet array and 555 kHz pulsed stimulation, the study reported downstream fMRI activity changes in primary visual cortex (E. Martin et al., 2025a).

Hippocampus

A second major line of work has focused on the hippocampus. In healthy mice, hippocampal sonication increased theta power, whereas in temporal-lobe epilepsy mice, stimulation with lower DC and PRF delayed seizure onset and shortened seizure duration (H. Yang et al., 2020). In humans, Brinker et al. reported a first-in-human, single-participant feasibility study in drug-resistant temporal-lobe epilepsy, demonstrating that pulsed low-intensity tFUS could be repeatedly delivered to hippocampal targets across multiple treatment sessions without reported adverse events (Brinker et al., 2020). Alzheimer's disease studies targeted the right hippocampus reported no detectable blood brain barrier (BBB) opening, yet found increased hippocampal glucose metabolism together with modest short-term memory improvement (Jeong et al., 2021, 2022).

Other subcortical regions

The field has also expanded into a broader set of deep targets. In macaques, offline sonication of the amygdala and ACC using a 250 kHz protocol reversibly altered the connectivity of the stimulated region for more than an hour (Folloni et al., 2019). An early human translation study targeting bilateral nucleus accumbens in patient with

opioid-use disorder using a phased array system reported reductions in craving (Mahoney et al., 2023). Matched 650 kHz, 5% DC paradigms applied to the amygdala or entorhinal cortex resulted in selective increases in perfusion and altered connectivity within the targeted regions (Kuhn et al., 2023). Evidence for brainstem and other deep targets remains more limited: sonication directed to the superior colliculus increased blink-reflex excitability, whereas comparable effects were not observed when stimulation was aimed at the substantia nigra or nucleus raphe magnus (Guerra et al., 2021).

2.4. Ultrasound transmission through the skull

The neuromodulation literature reviewed above suggests that tFUS can modulate neural activity with relatively high spatial selectivity, but also shows that its effects depend strongly on acoustic exposure parameters. This parameter dependence raises a critical translational question in human studies: although the acoustic output of the transducer can be predefined and controlled, this does not guarantee that the intended pressure, intensity or focal distribution is delivered accurately to the region of interest (RoI) after propagation through the head. Uncertainty in human tFUS is therefore not limited to waveform selection, but extends to the more fundamental question of whether the planned acoustic exposure corresponds to the in-situ exposure at the target.

From the scalp to the brain, the ultrasound beam must traverse several anatomical layers, including the scalp, skull, and meninges, before reaching neural tissue. The scalp and meninges are relatively thin soft-tissue structures compared with the skull, and their acoustic properties are broadly similar to those of other soft tissues. By contrast, the skull is a dense, heterogeneous, multi-layered bony structure with marked regional and inter-individual variability in thickness, composition, and internal architecture. As a result, the skull is the dominant source of transmission loss and beam distortion in tFUS. This problem becomes especially important for deep targets, where sufficient energy must pass through the skull while preserving adequate focal coherence within the brain.

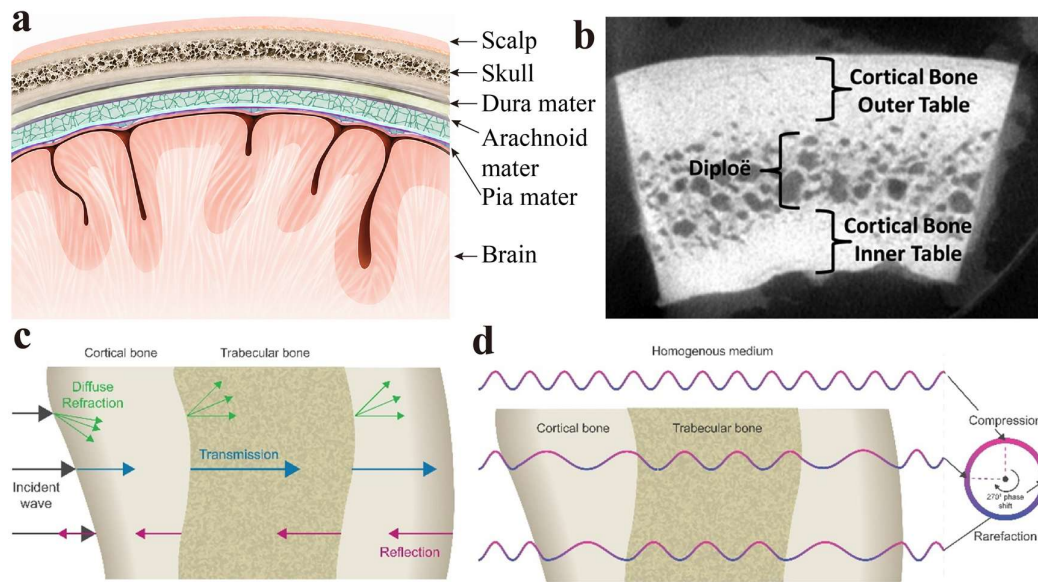


Figure 2-4 Anatomical and acoustic factors governing transcranial ultrasound transmission through the human skull. (a) Illustration showing the anatomical layers traversed by an ultrasound beam before reaching neural tissue. (b) Micro-CT illustration of the layered cranial bone structure, including the outer cortical table, trabecular diploë and inner cortical table. (c) Schematic representation of reflection, transmission and mode conversion at a bone interface. (d) Illustration of phase aberration caused by spatially varying sound speed across bone, showing how waves that begin in phase can emerge with different phase delays after transmission through the skull, thereby distorting the transmitted field and reducing focal coherence. Panels (b), (c) - (d) are adapted from (Lillie et al., 2016) and (Murphy et al., 2025) respectively.

2.4.1. Role of the cranial bone

The importance of the skull in brain ultrasound applications has been recognised for decades. Early interest in transcranial ultrasound was closely linked to brain imaging, where successful image formation required ultrasound energy enter the cranial cavity, reflect from intracranial structures and return through the skull to the transducer. This double passage through bone imposed a major limitation on image quality and feasibility. In therapeutic and neuromodulatory applications, the requirement is less restrictive because the primary objective is to deliver energy into the brain rather than recover reflected echoes. Even so, transcranial ultrasound remains fundamentally constrained by the acoustic properties of the skull.

This constraint is well illustrated in clinical MR-guided focused ultrasound (MRgFUS). In procedures such as MRgFUS thalamotomy for essential tremor, inter-individual

differences in skull acoustic properties have been associated with insufficient temperature rise at the intended target (Chang et al., 2016; Elias et al., 2016; Jung et al., 2019). Although the therapeutic goals and exposure regimes of MRgFUS differ from those of low-intensity neuromodulation, the same general principle applies: the skull does not behave as a passive window, but as a subject-specific filter that can substantially alter the transmitted field.

At the most basic level, transmission is limited by the acoustic impedance mismatch between soft tissue and bone. At the outer skull surface, this mismatch causes a substantial fraction of the incident wave to be reflected (roughly 58%) before energy can enter the skull (D. N. White et al., 1978). Once ultrasound enters bone, propagation becomes more complex because bone is a solid elastic medium in which mechanical energy can propagate not only as longitudinal waves but also as shear waves. The relative contribution of these modes depends strongly on the angle of incidence. As the incident beam becomes more oblique, a greater proportion of energy can be redistributed through reflection, refraction and mode conversion, reducing the forward-propagating longitudinal component and altering the resulting focal field (Clement et al., 2004).

Propagation is further complicated by the layered structure of the calvarium, which is typically composed of outer and inner cortical tables separated by trabecular diploë. These layers differ in density, porosity, and structural organisation, and each interface can introduce additional reflection, refraction, and mode conversion. Cortical bone is relatively compact, whereas trabecular bone has a porous cancellous architecture containing marrow and vascular spaces, with bone-soft tissue volume ratio about 50% to 50% (Boruah et al., 2015). Its internal microstructure is irregular, with trabeculae distributed over sub-millimetre to millimetre length scales (Collins et al., 2019) and separated from half to two millimetres in distance (Chaffai et al., 2000). At lower ultrasound frequencies, where the wavelength is longer, fine-scale trabecular heterogeneity may be partially averaged by the propagating wave. At higher frequencies (e.g., 1.5 mm at 1 MHz) however, the wavelength becomes comparable to skull microstructural features, increasing scattering and frequency-dependent attenuation. In this sense, the skull behaves as a complex frequency-dependent filter, and the

transmitted field depends not only on acoustic frequency but also on local skull thickness, internal composition, and beam incidence angle (Pinton et al., 2011). These factors help explain why tFUS outcomes can vary across individuals when studies rely on a single generic skull attenuation coefficient or assume a uniform cranial window for all participants (e.g., Sanguinetti et al., 2020a).

2.4.2. Targeting

In some applications, sonication may be delivered over a relatively broad region, particularly when the aim is to influence distributed pathology rather than a single circumscribed structure (e.g., Shimokawa et al., 2022). More commonly, however, tFUS neuromodulation aims to modulate a defined cortical or subcortical region. This requires the transducer to be positioned such that its acoustic focus overlaps with the intended target. The most straightforward approach uses a single geometrically focused transducer aligned according to the predefined target location. This approach is attractive because it is relatively simple to implement, but it assumes that the beam propagates through the skull in a predictable line-of-sight manner. Nevertheless, a practical challenge is that anatomical targeting and acoustic targeting are not necessarily equivalent. A transducer may be positioned correctly with respect to scalp landmarks or image-guided coordinates, yet still generate a degraded intracranial field if the beam passes through thicker, more heterogeneous or more obliquely oriented cranial regions.

For this reason, sonication is often preferentially directed through more acoustically favourable skull regions where transmission is less severely degraded. However, the ideal acoustic window may not coincide with the most direct geometric path to the neural target. Multi-element transducers provide a more flexible solution to this problem. By independently controlling the phase and amplitude of each element, phased-array systems can electronically steer and shape the acoustic field, allowing the focus to be directed towards a chosen target even when the array is not aligned along a simple perpendicular path. Such systems can also compensate, at least in part, for skull-induced phase aberration (Adams et al., 2021).

The geometry of the focal zone is also important. A single-element focused transducer typically produces an elongated focal region rather than an isotropic point focus. One strategy to reduce focal volume is to intersect two beams from separate transducers, for example in an orthogonal arrangement, so that the overlap region receives the highest pressure while the surrounding exposed volume is reduced (S. Kim et al., 2021). Extending this principle, a hemispherical distribution of elements can provide tighter focusing, albeit at the cost of greater system complexity (Leung et al., 2021). Such configurations also require larger scalp contact areas and more sophisticated acoustic coupling procedures (Gagliardo et al., 2018).

2.4.3. Characterisation

Because the skull can substantially alter ultrasound propagation, waves that would otherwise converge constructively at the intended focus may arrive with different phase delays (Figure 2-4.d), thereby reducing focal pressure or redistributing energy to unintended locations. These effects are difficult to measure directly in-vivo, where invasive pressure measurements are generally not feasible. As a result, much of the experimental characterisation of skull transmission has relied on ex-vivo measurements.

Underwater acoustic measurement is commonly used for this purpose. For a single-element transducer, a simple experiment compares the acoustic pressure measured in free water with the pressure measured after introducing a skull specimen into the propagation path, allowing transmission loss to be estimated. A more informative approach is to scan a cross-sectional or volumetric field, thereby visualising the degree of beam distortion. For multi-element systems, individual elements can be activated sequentially and the corresponding propagation delays measured. These delay estimates can then be used to adjust firing times so that the transmitted waves arrive more synchronously at the intended focus.

The choice of acoustic sensor depends on the expected exposure conditions and the required spatial resolution. Hydrophone-based measurements remain widely used because they provide direct pressure measurements with high temporal resolution. Sensitive polyvinylidene fluoride (PVDF) hydrophones are suitable for many low-intensity measurements, whereas more robust sensors, including fibre-optic

hydrophones, may be preferable under higher-intensity conditions where conventional probes may be vulnerable to damage. The sensor itself must also be chosen carefully, because finite aperture size, angular sensitivity and positioning accuracy can influence the measured field, particularly near sharp foci or in distorted transcranial fields.

2.4.4. Computational modelling

In practice, computational modelling serves as the bridge between experimentally observed skull behaviour and subject-specific treatment planning. A first requirement is adequate representation of skull anatomy. Computed tomography (CT) is often regarded as the most informative imaging modality for this purpose because it captures cranial morphology and bone contrast directly. MRI, by contrast, is intrinsically less suited to direct bone characterisation because bone contains relatively little mobile hydrogen and therefore generates weak signal. However, MRI remains attractive in clinical contexts because it is non-ionising, motivating increasing interest in generating synthetic CT (sCT) bone information from MRI using deep-learning approaches (Xiao et al., 2025).

Once the skull has been represented anatomically, the transducer and sonication conditions must also be modelled appropriately. Commercial platforms such as Sim4life (Kosnoff et al., 2026) provide integrated environments for treatment planning, ray-based approaches such as Kranion (Sammartino et al., 2019) can provide relatively efficient estimates of propagation and phase correction, and full-wave solvers such as k-Wave (Treeby & Cox, 2010) gives larger freedom in modelling acoustic interference and heterogeneous propagation, to name but a few.

Nevertheless, the accuracy of any simulation depends heavily on the quality of its inputs. Errors in skull segmentation, material-property assignment, transducer geometry, or target registration can all produce misleading estimates of the in-situ field. These uncertainties are central to the following chapters, which examine how transcranial acoustic delivery can be characterised, modelled and evaluated.

2.4.5. Monitoring and validation

For high-intensity applications that deliberately produce thermal effects, MR thermometry can provide spatial information about temperature elevation and is therefore a powerful monitoring tool. In low-intensity neuromodulation, by contrast, appreciable heating is typically neither intended nor desirable, so thermal imaging is often insufficient as a marker of delivered acoustic dose or target engagement. One possible alternative is magnetic resonance acoustic radiation force imaging (MR-ARFI), which measures the small tissue displacements induced by acoustic radiation force and can therefore provide indirect evidence of focal energy deposition (Mohammadjavadi et al., 2025). Tracker-based navigation systems provide another practical tool by monitoring transducer position and orientation relative to the planned target, particularly in experimental setups requiring mobility or iterative adjustment (Shin et al., 2023).

A further level of validation comes from monitoring neural or physiological responses. Techniques such as EEG, EMG, and fMRI are commonly used to assess whether sonication produces measurable changes in brain or motor activity, although the observed response may indicate that ultrasound has influenced the nervous system, yet it does not by itself confirm that the effect arose from the intended focal region rather than from off-target exposure, auditory confounds, or network-level secondary effects.

3. Acoustic source characterisation

The purpose of this chapter is to establish the physical and experimental framework used to characterise the ultrasound sources employed throughout this thesis. The electromechanical behaviour of the piezoelectric elements is first described using the linear constitutive relations and a reduced thickness-mode description linking electrical excitation to ceramic displacement. Source motion is then related to measurable acoustic output through acoustic radiation force, intensity and power. The chapter subsequently presents the experimental methods used to quantify these quantities, including laser Doppler vibrometer, radiation force balance measurements and hydrophone-based pressure-field mapping. Finally, acoustic holography is introduced as a method for recovering the amplitude and phase structure of the emitted field. Together, these approaches define the source-characterisation framework underpinning the modelling, validation and transcranial ultrasound studies described in the subsequent chapters.

3.1. Piezoceramics

Four single-element concave transducers were employed in this project. Each had an aperture diameter of 60 mm and a radius of curvature (RoC) of 75 mm, with nominal centre frequencies of 220 kHz, 650 kHz, 680 kHz, and 1000 kHz. The selected frequencies cover the sub-MHz range relevant to transcranial ultrasound neuromodulation and enable investigation of the fundamental trade-off between skull transmission and focal confinement. These transducers served as the acoustic sources for the measurement, modelling, and validation studies presented in this thesis.

3.1.1. Linear constitutive relations

Piezoelectric ceramics convert electrical excitation into mechanical deformation through the inverse piezoelectric effect, whereby an applied electric field induces strain. This electromechanical coupling underpins acoustic wave generation in ultrasound transducers. When a piezoceramic is driven by an applied voltage, an electric field (E , V/m) is established inside the body. The electromechanical response is described by constitutive relations that link the local electrical and mechanical field: the electric displacement (D , C/m²), the strain tensor (S), and the stress tensor (T , Pa). For piezo materials with simple geometrical shapes, mechanical and electrical dissipation was theoretically neglected, the stress-charge form reads (*IEEE Standard on Piezoelectricity*, 1987)

$$T_{ij} = C_{ijkl}^E S_{kl} - e_{kij} E_k, \quad D_i = e_{ikl} S_{kl} + \varepsilon_{ik}^S E_k. \quad (3.1.1)$$

Where C_{ijkl}^E are the elastic stiffness at constant field (Pa), e_{kij} are the piezoelectric stress-charge coefficients (C·m⁻²) and ε_{ik}^S are the dielectric permittivity constants (F·m⁻¹). Worth noting that, because stress and strain are symmetric, conventionally the symmetric index pairs (ij or kl : 11, 22, 33, 23, 13, 12) is packed using Voigt notation (1 to 6). With this mapping, the C_{ijkl}^E , e_{kij} and ε_{ik}^S becomes 6×6 and 3×6 and 3×3 matrices, respectively. The equivalent strain-charged form and conversion can be found

at (Erturk & Inman, 2011). Although it appears more common to derive from strain-charge, in this chapter we proceed from stress-charge. Either form is sufficient to define the linear elastic-electric vibration of a given piezoceramic.

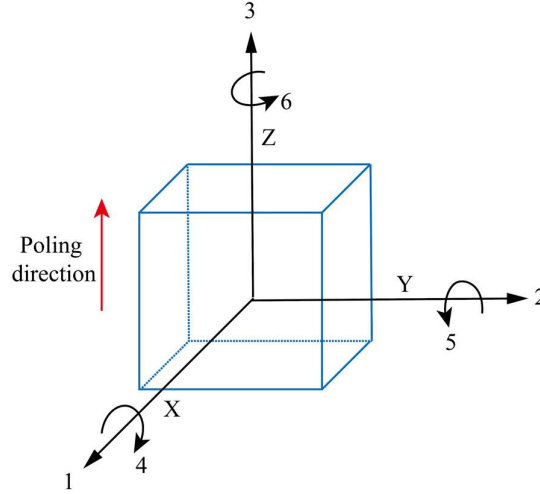


Figure 3-1 Coordinate axes and poling direction for an infinitesimal piezoelectric element.

For prolonged, high-power applications, lead zirconate titanate (PZT) with additional acceptor doping is commonly preferred because such hardening process suppresses domain-wall motion, increases coercive field strength, and reduces dielectric and mechanical losses (Jakes et al., 2011). The transducers used in this project employ two specific types of hard PZT: PZ54 and DL-47. The stress-charged constants obtained from manufacturer are summarized in Table 3-1. Together with density and mechanical quality factor, these parameters define the linear electromechanical response of the ceramic.

Table 3-1: Elastic-electric constants of PZ54 and DL-47, compiled from Qiu's Thesis and manufacturer data (Qiu, 2014).

	Elastic stiffness constants, C^E (10^{10}N/m^2)					
	C^E_{11}	C^E_{12}	C^E_{13}	C^E_{33}	C^E_{55}	C^E_{66}
PZ54	12.1	6.7	5.8	11.9	2.9	2.7
DL-47	15.6	8.9	8.8	13.2	3.1	3.4
	Piezoelectric stress coefficients, e (C/m^2)					
	e_{33}, e_{32}	e_{31}		e_{15}, e_{24}		
PZ54	21.6	-5.6		11.7		
DL-47	18.5	-4.9		14.3		
	Relative dielectric permittivity, ϵ^S					
	ϵ^S_{33}			ϵ^S_{11}		

PZ54	1441	1220
DL-47	870	960

3.1.2. Reduced thickness-mode model

To obtain an analytical description of the ceramic motion, consider an axisymmetric piezoceramic plate uniformly poled along the axial (3-axis, Figure 3-1) and driven predominantly in thickness mode. Apply a voltage establishes an electric field E_3 through the thickness. Under a lateral stress-free approximation, shear components are neglected and only the normal stresses and strains are retained. The reduced stress-charge relations are

$$\begin{bmatrix} T_1 \\ T_2 \\ T_3 \end{bmatrix} = \begin{bmatrix} C_{11} & C_{12} & C_{13} \\ C_{12} & C_{11} & C_{13} \\ C_{13} & C_{13} & C_{33} \end{bmatrix} \begin{bmatrix} S_1 \\ S_2 \\ S_3 \end{bmatrix} - \begin{bmatrix} e_{31} \\ e_{31} \\ e_{33} \end{bmatrix} E_3. \quad (3.1.2)$$

This yields the scalar relations:

$$T_1 = C_{11}S_1 + C_{12}S_2 + C_{13}S_3 - e_{31}E_3, \quad (3.1.3)$$

$$T_2 = C_{12}S_1 + C_{11}S_2 + C_{13}S_3 - e_{31}E_3, \quad (3.1.4)$$

$$T_3 = C_{13}S_1 + C_{13}S_2 + C_{33}S_3 - e_{33}E_3. \quad (3.1.5)$$

For traction-free lateral faces, $T_1 = T_2 \approx 0$. Subtracting (3.1.3) and (3.1.4) gives

$$(C_{11} - C_{12})(S_1 - S_2) = 0. \quad (3.1.6)$$

And since $C_{11} \neq C_{12}$ for the materials used here, it follows that $S_1 = S_2 = \frac{e_{31}E_3 - C_{13}S_3}{C_{11} + C_{12}}$.

Substituting this result back into (3.1.5) and yields an effective 1-D relation

$$T_3 = \left(C_{33} - \frac{2C_{13}^2}{C_{11} + C_{12}} \right) S_3 + \left(\frac{2C_{13}e_{31}}{C_{11} + C_{12}} - e_{33} \right) E_3. \quad (3.1.7)$$

Which can also be written in the strain-charge form:

$$S_3 = S_{33}^E T_3 + d_{33} E_3, \quad (3.1.8)$$

$$S_{33}^E = \frac{C_{11} + C_{12}}{(C_{11} + C_{12})C_{33} - 2C_{13}^2}, \quad d_{33} = \frac{e_{33}(C_{11} + C_{12}) - 2C_{13}e_{31}}{(C_{11} + C_{12})C_{33} - 2C_{13}^2}. \quad (3.1.9)$$

Where S_{33}^E is the effective thickness compliance (m^2/N) and d_{33} is the effective piezoelectric strain coefficient (m/V). Using the constants in Table 3-1 gives $d_{33} \approx 3.0 \times 10^{-10} \text{ m/V}$ for PZ54 and $3.2 \times 10^{-10} \text{ m/V}$ for DL-47. For a monolithic plate of thickness h driven by voltage V , the free stroke Δh is therefore

$$\Delta h = d_{33}V. \quad (3.1.10)$$

Using the calculated d_{33} values, a 10 Vpp drive corresponds to a non-resonant free displacement of approximately 1.5 nm for PZ54 and 1.6 nm for DL-47.

3.1.3. Resonant behaviour

When the ceramic is driven at its natural frequency, the material behaves as a harmonic oscillator in which the kinetic energy and elastic strain energy shuttles back and forth each quarter period (Erhart et al., 2017), the energy accumulated through cycles of construction, and the achievable amplification is related to the mechanical quality factor, ($Q_m > 1000$ for PZ54 and DL-47). For the fundamental thickness-extensional mode (diameter $\gg$ thickness), The resonant frequency can be approximated by

$$f_s = \frac{N_t}{h}, \quad (3.1.11)$$

where N_t is the thickness frequency constant ($\text{Hz}\cdot\text{m}$), which is approximately half the longitudinal wave speed in the ceramic. Reference values of N_t for PZ54 and DL-47 are about 1980 $\text{Hz}\cdot\text{m}$ and 2130 $\text{Hz}\cdot\text{m}$, respectively. This relation provides a first estimate of ceramic thickness for a chosen operating frequency. In practice however, the response of an assembled transducer is not determined by the ceramic alone. The housing constraints, electrode layout, and manufacturing tolerances all modify the effective resonance frequency and vibration amplitude.

The surface displacement of selected transducers was measured in air using a laser Doppler vibrometer (LDV; OFV-2570, Polytec, DE). As shown in Figure 3-2, The laser was aligned with the centre of the ceramic surface to maximise direct reflection, and the displacement decoder was sampled at up to 2 MHz. Three transducers with nominal

centre frequencies (f_c) of 220 kHz, 680 kHz and 1000 kHz were driven with a 10 V_{pp} sinusoidal waveform.

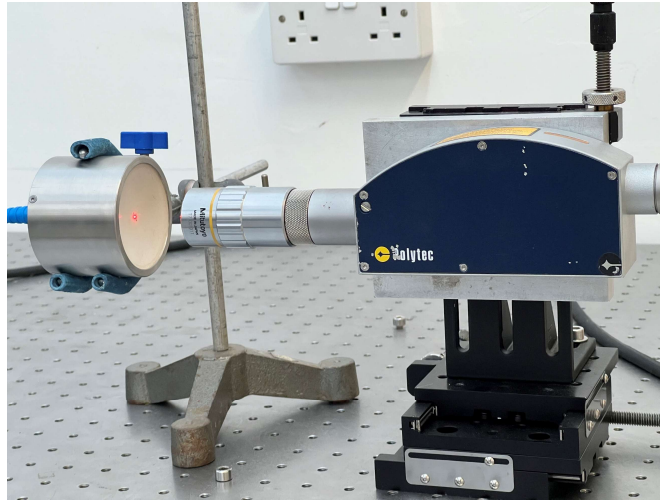


Figure 3-2 Piezoceramic surface displacement measurement setup using LDV.

The measured peak displacements occurred at operating frequencies (f_o) of 226 kHz, 690 kHz, and 1027 kHz, respectively, with average peak amplitudes of 36.6 nm, 20.9 nm, and 24.0 nm (Figure 3-2). Relative to the analytical non-resonant free-stroke estimates, these measured amplitudes were approximately 24, 14, and 15 times larger, consistent with substantial resonant amplification. The slight shift between nominal and measured operating frequency is expected because the complete transducer structure, rather than the piezoceramic alone, determines the effective resonance.

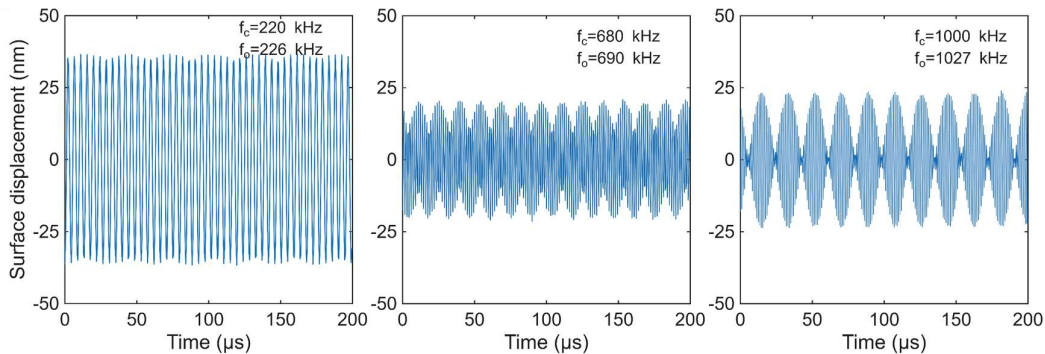


Figure 3-3 Measured surface displacement in three transducers with centre frequencies (f_c) of 220 kHz, 680 kHz and 1000 kHz. The peak displacements occurred at operating frequencies (f_o) of 226 kHz, 690 kHz and 1027 kHz, respectively.

One important limitation should be noted. For the 1027 kHz measurement, the maximum sampling rate of 2 MHz was slightly below the Nyquist requirement, so the recorded waveform at the highest frequency was distorted and the peak amplitude may

be biased. In the present work, the transducers were commercially manufactured devices for which the detailed internal stack geometry, material properties and bonding conditions were not available. A sufficiently representative finite-element model could therefore not be constructed without introducing substantial assumptions. Consequently, the transducer response was characterised experimentally rather than predicted from a device-level piezoelectric simulation

3.1.4. Pressure gain estimates

For a harmonic source with peak displacement amplitude Δh , the corresponding peak normal surface velocity (v_0 , m/s) can be approximated as

$$v_0 = \omega \Delta h, \quad (3.1.12)$$

where $\omega = 2\pi f$. Under a lossless plane-wave approximation, this velocity corresponds to plane-wave-equivalent pressure amplitude (p_0 , Pa),

$$p_0 = v_0 \rho c, \quad (3.1.13)$$

where ρ is the density of the coupling medium (kg/m^3) and c is the speed of sound (m/s).

It is important to note that the surface displacement in this study was measured in air. This represents a lightly loaded condition and does not reproduce the acoustic loading experienced during water-coupled operation, where the higher acoustic impedance of water imposes a substantially greater radiation load on the transducer surface. Direct measurement of the water-loaded displacement was not obtained, because refraction and scattering of the LDV beam at the water-container interface degraded the reflected optical signal. Therefore, the air-measured displacement was not used to calculate calibrated acoustic pressures. Instead, the following relations are used only to show how pressure gain scales with surface velocity and focusing geometry.

For an ideal spherical concave radiator vibrating with uniform normal velocity, the Rayleigh-O'Neil solution gives the complex on-axis pressure at the geometrical focus as (O'Neil, 1949)

$$\hat{p}(F) = i\rho c v_0 k \frac{S}{2\pi F} e^{-ikF}, \quad (3.1.14)$$

where F is the geometrical focal length, $k = 2\pi/\lambda$ is the wavenumber; λ is the wavelength, $S = \pi r^2$ is the projected aperture area of the source with aperture radius r . Taking the magnitude at the focus gives

$$|\hat{p}(F)| = \rho c v_0 \frac{kr^2}{2F}. \quad (3.1.15)$$

Dividing $|\hat{p}(F)|$ by p_0 gives the focal pressure gain,

$$G_p \equiv \frac{\pi r^2}{F\lambda}. \quad (3.1.16)$$

For the 60 mm aperture and 75 mm RoC transducers used here, and using the wavelength in water, the predicted pressure gains are approximately 5.5, 17.1 and 25.3 at 220 kHz, 680 kHz, and 1000 kHz, respectively.

3.2. Energy characterisation

3.2.1. Momentum flux expression

In this section, the relation between piezoelectric surface motion and measured acoustic output is established. The oscillatory displacement of the piezoceramic radiating surface imposes a normal boundary velocity on the adjacent coupling medium, thereby generating first-order perturbations in acoustic pressure (p), density (ρ), and particle velocity ($\mathbf{v}$). Neglecting viscosity and body forces, the momentum balance of the fluid is governed by Euler's equation,

$$\rho \left(\frac{\partial \mathbf{v}}{\partial t} + (\mathbf{v} \cdot \nabla) \mathbf{v} \right) + \nabla p = 0 \quad (3.2.1)$$

Applying the product rule gives

$$\rho \frac{\partial \mathbf{v}}{\partial t} = \frac{\partial(\rho \mathbf{v})}{\partial t} - \mathbf{v} \frac{\partial \rho}{\partial t}, \quad \rho \mathbf{v} \cdot \nabla \mathbf{v} = \nabla \cdot (\rho \mathbf{v} \mathbf{v}) - \mathbf{v} \nabla \cdot (\rho \mathbf{v}),$$

where $\mathbf{v} \mathbf{v}$ denotes the dyadic product. Euler's equation may therefore be written as

$$\frac{\partial(\rho \mathbf{v})}{\partial t} + \nabla \cdot (\rho \mathbf{v} \mathbf{v}) - \mathbf{v} \left(\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{v}) \right) + \nabla p = 0. \quad (3.2.2)$$

Using the continuity equation for mass conservation,

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{v}) = 0, \quad (3.2.3)$$

Eq. (3.2.2) reduces to the conservative momentum form,

$$\frac{\partial(\rho \mathbf{v})}{\partial t} + \nabla \cdot (p \mathbf{I} + \rho \mathbf{v} \mathbf{v}) = 0, \quad (3.2.4)$$

where $\mathbf{I}$ is the identity tensor. Integrating Eq. (3.2.4) over a control volume V gives

$$\frac{d}{dt} \int_V \rho \mathbf{v} dV + \iint_{\partial V} (p \mathbf{I} + \rho \mathbf{v} \mathbf{v}) \cdot \mathbf{n} dA = 0, \quad (3.2.5)$$

where $\mathbf{n}$ is the outward unit normal of the control volume. For periodic steady state, the cycle average over one acoustic cycle of the first term vanishes, such that

$$\iint_{S_I} \langle (p \mathbf{I} + \rho \mathbf{v} \mathbf{v}) \cdot \mathbf{n} \rangle dA = - \iint_{S_O} \langle (p \mathbf{I} + \rho \mathbf{v} \mathbf{v}) \cdot \mathbf{n} \rangle dA, \quad (3.2.6)$$

where ∂V includes S_O the outer surface and S_I the inner surface surrounding the body. Defining the momentum-flux tensor as

$$\mathbf{T} = p \mathbf{I} + \rho \mathbf{v} \mathbf{v},$$

the radiation force on the body is obtained from the flux of the cycle-averaged momentum-flux tensor across the inner surface,

$$F_{rad} = - \iint_{S_I} \langle \mathbf{T} \rangle \cdot \mathbf{n} dA, \quad (3.2.7)$$

To evaluate $\mathbf{T}$, the coupling medium is treated as an inviscid, compressible fluid under the isentropic small-perturbation approximation. The field variables are expanded about a quiescent equilibrium state as

$$\begin{aligned}
p &= p_0 + \varepsilon p_1 + \varepsilon^2 p_2 + \cdots, \\
\rho &= \rho_0 + \varepsilon \rho_1 + \varepsilon^2 \rho_2 + \cdots, \\
\mathbf{v} &= \varepsilon \mathbf{v}_1 + \varepsilon^2 \mathbf{v}_2 + \cdots.
\end{aligned} \tag{3.2.8}$$

Where p_0 and ρ_0 are the uniform equilibrium pressure and density, and ε is a small parameter representing the small acoustic perturbation amplitude with $\varepsilon \ll 1$. At first order, substitution of Eq. (3.2.8) into Eq. (3.2.1) yields

$$\rho_0 \frac{\partial \mathbf{v}_1}{\partial t} + \nabla p_1 = 0. \tag{3.2.9}$$

For a monochromatic plane progressive wave, the first-order pressure and particle velocity may be written as

$$p_1(\mathbf{r}, t) = \hat{p} e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)}, \quad \mathbf{v}_1(\mathbf{r}, t) = \hat{\mathbf{v}} e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)}, \tag{3.2.10}$$

Where $\hat{p}$ and $\hat{\mathbf{v}}$ are complex amplitudes, $\mathbf{r}$ is the position vector, and $\mathbf{k}$ is the wavevector and $|\mathbf{k}| = \omega/c_0$, with c_0 denoting the sound speed in the equilibrium medium. The physical pressure and particle velocity are given by the real parts of these expressions. Taking the longitudinal scalar component along the propagation direction gives the first-order pressure-velocity relation

$$v_1 = \frac{p_1}{\rho_0 c_0}, \tag{3.2.11}$$

where $\rho_0 c_0$ is the characteristic acoustic impedance of the medium.

The detailed second-order derivation is given in APPENDIX B, the resulting cycle-averaged second-order momentum-flux tensor is

$$\langle \mathbf{T}^{(2)} \rangle = \left\langle \frac{p_1^2}{2\rho_0 c_0^2} - \frac{\rho_0 v_1^2}{2} \right\rangle \mathbf{I} + \rho_0 \langle \mathbf{v}_1 \mathbf{v}_1 \rangle. \tag{3.2.12}$$

For a plane progressive wave, the cycle-averaged isotropic second-order term vanishes while $\rho_0 \langle \mathbf{v}_1 \mathbf{v}_1 \rangle$ remains, and substituting Eq. (3.2.11) into Eq. (3.2.7) therefore gives

$$F_{rad} = \iint_{S_I} \left(\frac{p_{1,rms}^2}{\rho_0 c_0^2} \right) dA, \tag{3.2.13}$$

where $p_{1,rms}$ is the root-mean-squared first-order acoustic pressure.

The time-averaged acoustic intensity is

$$I = \langle p_1 \mathbf{v}_1 \rangle = \frac{p_{1,rms}^2}{\rho_0 c_0}, \quad (3.2.14)$$

integration of the intensity over the beam cross-section gives the total acoustic power,

$$P = \iint_A I dA. \quad (3.2.15)$$

For normal incidence on an ideal absorbing target, the measured radiation force is related to the total acoustic power by

$$F_{rad} = \frac{P}{c_0}. \quad (3.2.16)$$

The detailed first-order pressure-velocity relation and intensity expression are derived in APPENDIX C.

3.2.2. Power measurement

Following the radiation-force and intensity relations derived in the preceding subsection, acoustic power was quantified experimentally using a radiation force balance (RFB; Precision Acoustics, UK), following IEC 61161 as closely as practicable.

For a monochromatic plane progressive wave normally incident on an ideal absorbing target, the time-averaged radiation force is proportional to the square of the root-mean-squared acoustic pressure, thereby providing the theoretical link between the first-order acoustic field generated by the vibrating piezoceramic surface and the measurable force exerted on the absorber.

As shown in Figure 3-4.a, the transducer was immersed in degassed water and aligned normal to the absorbing target. The absorber area exceeded the transducer aperture, and its position was chosen such that essentially all forward-propagating acoustic power was intercepted within the measurement geometry. The RFB recorded radiation force as an equivalent mass, which was converted to force using $F = mg$. Representative data

are shown in Figure 3-4.b for the 650 kHz transducer driven by a 10 Vpp continuous sinusoidal waveform. The measured mass signal was averaged over nine on-off cycles separated by 20 s intervals.

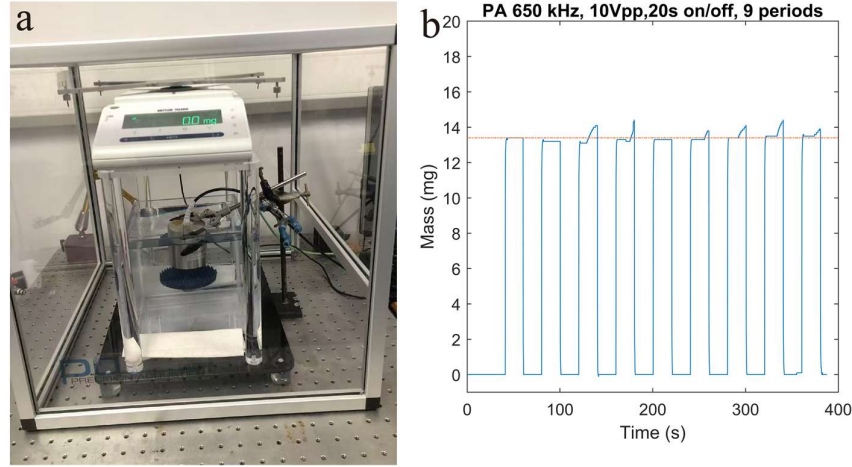


Figure 3-4. (a) Representative radiation-force measurement setup. The transducer was positioned normal to the absorber at a distance shorter than the focal depth. (b) Representative radiation-force measurement expressed as equivalent mass for a 650 kHz transducer driven by a 10 Vpp continuous sinusoidal waveform. The signal was averaged over nine on-off cycles with 20 s intervals.

The same method was then used to determine voltage-to-power conversion curves for the 220 kHz, 650 kHz, 680 kHz, and 1000 kHz transducers. Depending on the amplifiers used (E&I, US), the maximum available drive voltage ranged from 66 Vpp to 185 Vpp, and measurements were performed in increments of 0.3 to 1.5 Vpp. The resulting curves (Figure 3-5) show that acoustic power increased with excitation voltage for all transducers, whereas the electrical-to-acoustic conversion efficiency decreased at higher drive levels. Among the devices tested, the 220 kHz transducer exhibited the lowest conversion efficiency. Notably, the 650 kHz and 680 kHz transducers, despite their similar nominal centre frequencies, produced clearly different acoustic power outputs. This indicates that transducer performance depends not only on nominal frequency and external geometry, but also on internal stack design, material-specific properties, and assembly-related factors. These findings therefore reinforce the need to calibrate each transducer individually rather than assuming identical acoustic performance from nominal specifications alone.

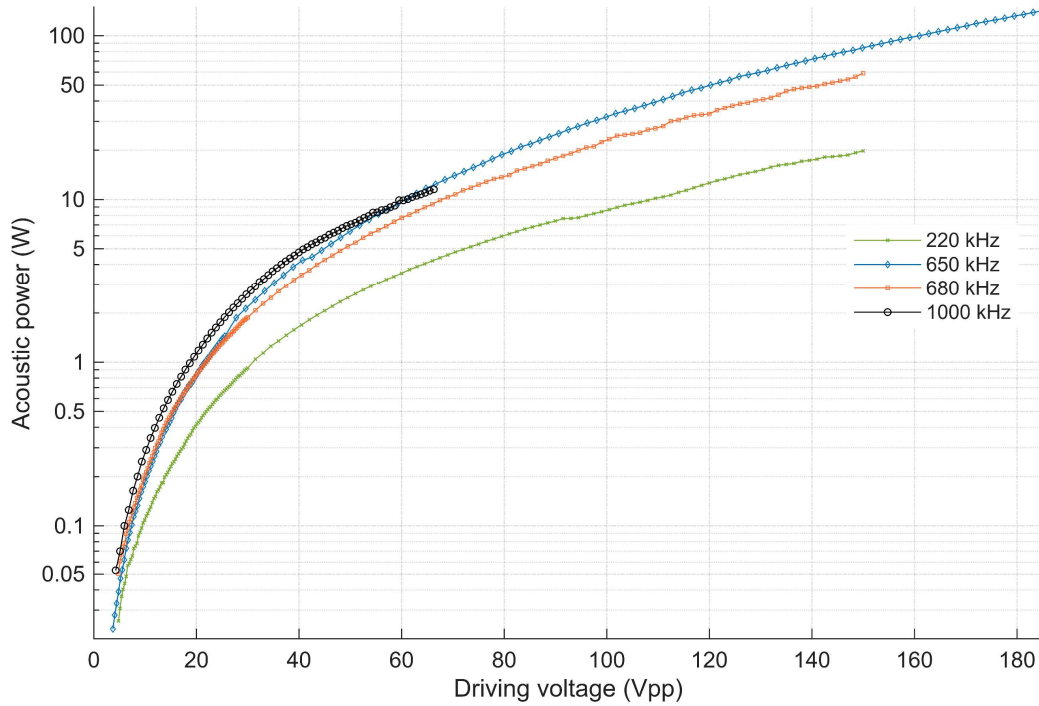


Figure 3-5. Electrical drive voltage-to-acoustic power conversion curves. Voltage ranges were 4.8-150 Vpp for the 220 kHz and 680 kHz transducers, 3.7-185 Vpp for the 650 kHz transducer, and 4.3-66.3 Vpp for the 1000 kHz transducer.

3.2.3. Pressure field measurement

Acoustic pressure fields were measured using PVDF needle hydrophones, which convert incident acoustic pressure into voltage through the piezoelectric effect. For high-resolution field mapping, smaller active-element diameters are advantageous because they reduce spatial averaging and directional error, although this occurs at the expense of lower sensitivity.

Measurements were performed in accordance with IEC 61828 and IEC 62127-1 to 3. A three-axis scanning system was assembled using Velmex XSlides (Velmex, USA) with a lead-screw resolution of 0.01 mm. In-house developed software enabled 1D, 2D, and 3D raster scanning with user-defined step sizes. The hydrophone was mounted in a 3D-printed holder incorporating an angled beam-splitter arrangement to reduce reflection artefacts.

Because the hydrophone records voltage rather than acoustic pressure directly, a frequency-dependent sensitivity is required to convert the measured signal to an

absolute pressure value. In this work, that sensitivity was established empirically by matching hydrophone-derived power estimates to RFB measurements at each working frequency.

The directional response of the hydrophones was first assessed under 650 kHz sonication by rotating the probe from 0° to 50° in 10° increment while maintaining the sensing tip at the geometrical focus. As shown in Figure 3-6, when the transducer was driven by a 10 Vpp continuous sinusoidal waveform, the received signal amplitude decreased with hydrophone diameter, reflecting the lower sensitivity of smaller active elements. Normal-incidence signal amplitudes, normalised to the 1.0 mm hydrophone, were 0.43, and 0.21 for the 0.5 mm, and 0.2 mm hydrophones, respectively.

The larger hydrophones also exhibited greater angular dependence. The average amplitude reduction per 10° was 6.1% for the 1.0 mm hydrophone, 4.6% for the 0.5 mm hydrophone, and only 0.4% for the 0.2 mm hydrophone. These results confirm the expected trade-off: smaller sensing tips provide improved spatial fidelity and lower directional bias, whereas larger tips provide stronger signal levels.

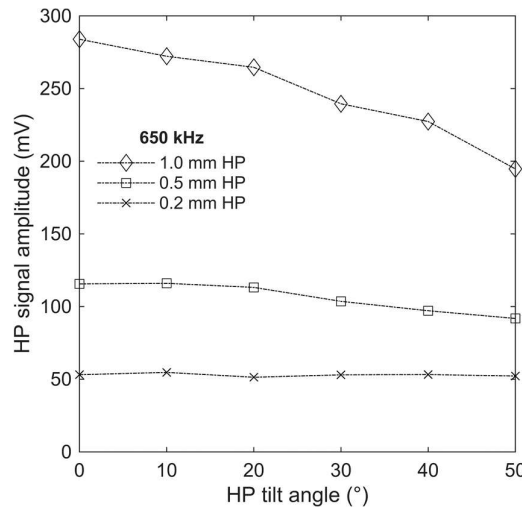


Figure 3-6 Relationship between hydrophone tilt angle and received signal amplitude under 650 kHz sonication.

To characterise the near field, the hydrophone was positioned 7 mm from the transducer aperture and scanned across a 66 mm × 66 mm plane, as illustrated in Figure 3-7. At each position, the transducer was driven by a 10 Vpp, 0.5 ms sinusoidal burst, and the waveform was recorded using a PC oscilloscope (4224A, Picoscope, US) at 40 MHz.

After a 0.5 s settling period, the hydrophone advanced to the next position. Initial transients and ring-down were excluded, and the steady-state component near the centre frequency was used to estimate peak signal amplitude.

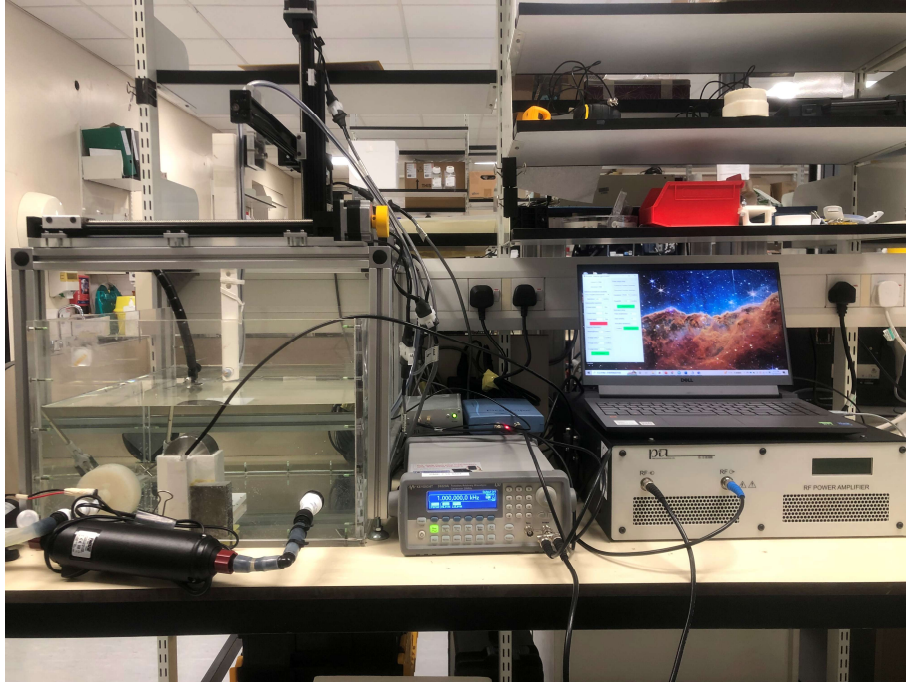


Figure 3-7 Acoustic scanning system setup.

To determine an appropriate balance between spatial resolution and acquisition time, three step sizes were evaluated: 1.0 mm, 0.5 mm, and 0.25 mm. Near-field maps for the 220 kHz, 680 kHz, and 1000 kHz transducers (Figure 3-8) showed that 1.0 mm sampling produced the coarsest field representation but required only approximately 2.0 hours, whereas 0.5 mm and 0.25 mm sampling produced similar field structures but required approximately 8.2 hours and 32.7 hours, respectively. Using the 0.25 mm scan as the reference, the integrated power differences for 1.0 mm and 0.5 mm sampling were +2.0% and -1.6% at 220 kHz, -3.9% and -1.3% at 680 kHz, and +35.6% and -2.6% at 1000 kHz. The larger discrepancy for 1.0 mm sampling at 1000 kHz is consistent with under-sampling of the finer spatial structure and steeper pressure gradients at the higher frequency, whereas the close agreement between the 0.5 mm and 0.25 mm scans indicates improved spatial convergence at the finer resolutions. These results indicate that 0.5 mm sampling provided a favourable compromise between spatial fidelity and measurement duration. Therefore, the selection of 0.5 mm was selected empirically by

its close agreement with the finer 0.25 mm scans together with the substantial reduction in acquisition time.

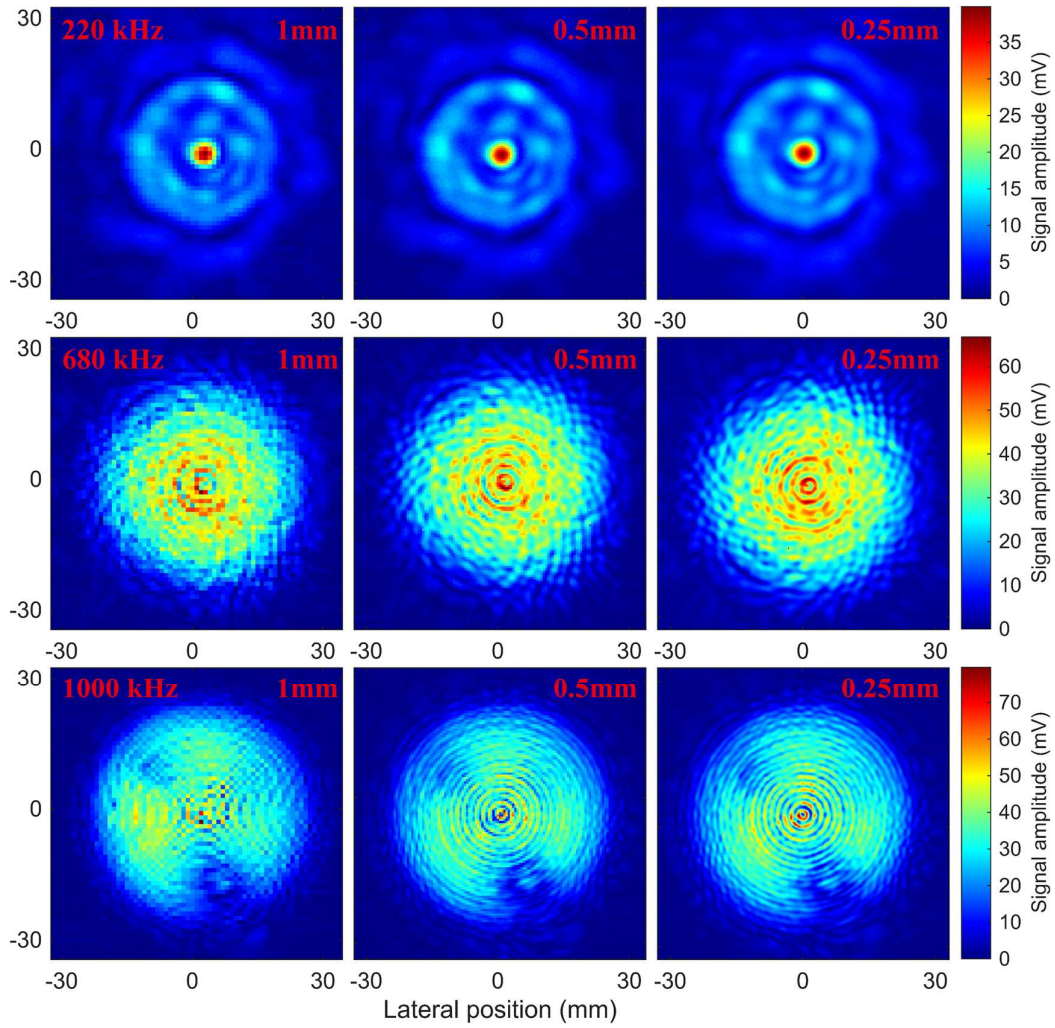


Figure 3-8 Near-field acoustic maps of the 220 kHz, 680 kHz, and 1000 kHz transducers acquired with step sizes of 1.0 mm, 0.5 mm, and 0.25 mm.

At near-focus distance, where the beam was narrower and the pressure distribution more concentrated, scans were performed over an area extending to at least twice the beam full-width half-maximum (FWHM). The measured hydrophone voltage field was then integrated to obtain a power estimate, and a frequency-specific amplitude-to-pressure conversion factor was derived by matching this power to the RFB-measured power over the range 3.7-16.3 V_{pp} in increments of 0.3 V_{pp}.

As shown in Figure 3-9, for the 650 kHz transducer, hydrophone-derived power agreed closely with RFB-derived power above approximately 8 Vpp. The larger deviations observed at lower voltages were most likely attributable to reduced hydrophone signal-to-noise ratio and the lower sensitivity of the RFB near its measurement threshold. This cross calibration procedure placed the hydrophone measurements on an absolute pressure scale and was subsequently used throughout the project to estimate peak pressure fields.

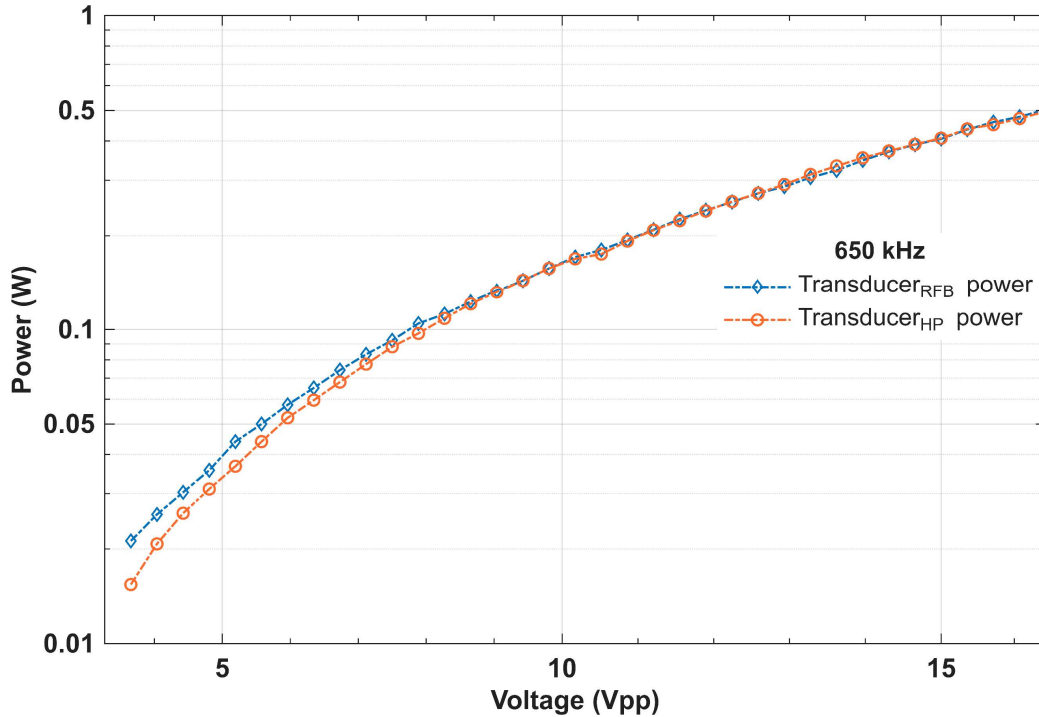


Figure 3-9 Comparison of acoustic power measured by the RFB and by hydrophone integration for the 650 kHz transducer.

3.2.4. Acoustic holography

The preceding subsection dealt primarily with pressure amplitude, since the excitation burst and hydrophone acquisition were synchronised, the full waveform recorded at each scan position also preserved phase information. This allowed the acoustic field to be represented as a complex pressure distribution and, in turn, enabled reconstruction of the three-dimensional acoustic field using the angular spectrum method (ASM) (Sapozhnikov et al., 2015).

In ASM, a measured two-dimensional monochromatic complex field is decomposed into its constituent spatial-frequency components and propagated to an arbitrary plane in the frequency domain. In this way, the field at different axial positions can be reconstructed from a single measured plane. In the present work, this propagation was implemented using the *angularSpectrumCW* function from the k-Wave pseudo-spectral MATLAB toolbox.

Before reconstructing the three-dimensional acoustic field, a holographic comparison was first performed between an ideal k-Wave-generated bowl-source hologram and the experimentally measured near-field signal, both viewed at the source aperture plane. As shown in Figure 3-10, the measured fields exhibited asymmetric pressure distributions and weaker contrast than the idealised source, particularly at 220 kHz. This behaviour is likely to reflect non-uniform vibration across the piezoelectric aperture, which may become more pronounced in thicker ceramics and at lower operating frequencies.

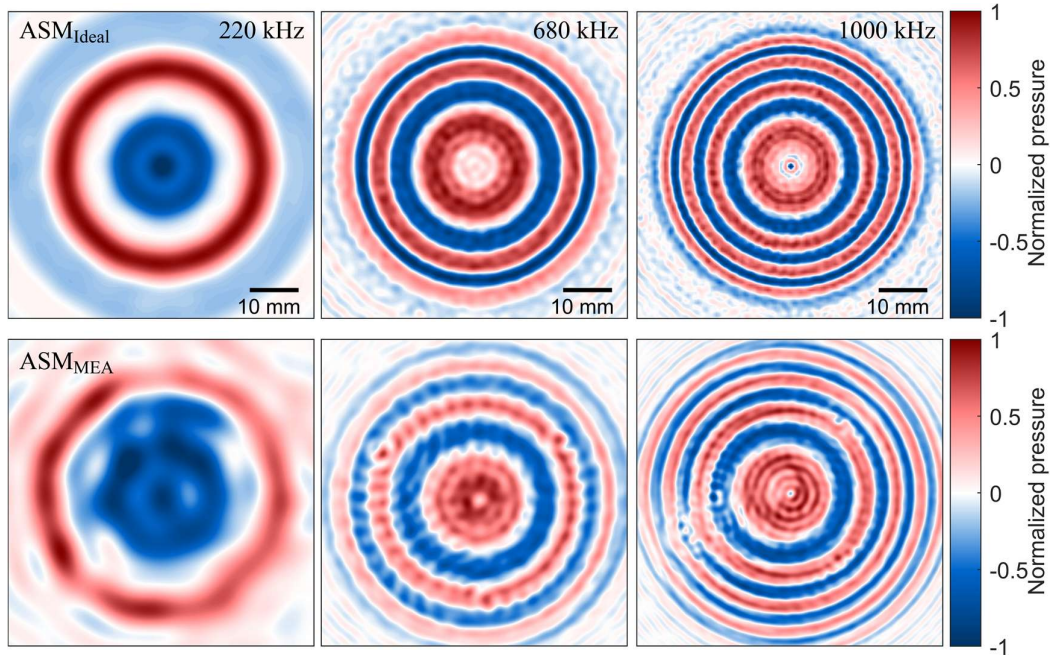


Figure 3-10 ASM-based plane propagation of the ideal k-Wave-generated bowl source and the measured signal, viewed at the source aperture plane. Pressure levels are normalised to their respective maxima.

To recover the effective bowl-surface source from measurement, rather than relying on an idealised geometrical source, the measured hologram was back-propagated to a bowl detector using the conventional k-space solver *kspaceFirstOrder3D*. The recorded

pressure on this detector was then taken as the transducer surface pressure field. This reconstruction allowed the effective source structure to be visualised directly and also provided a measurement-informed source definition for subsequent transcranial simulations. The reconstructed source field was then forward propagated to generate the free-field acoustic volume used for source validation.

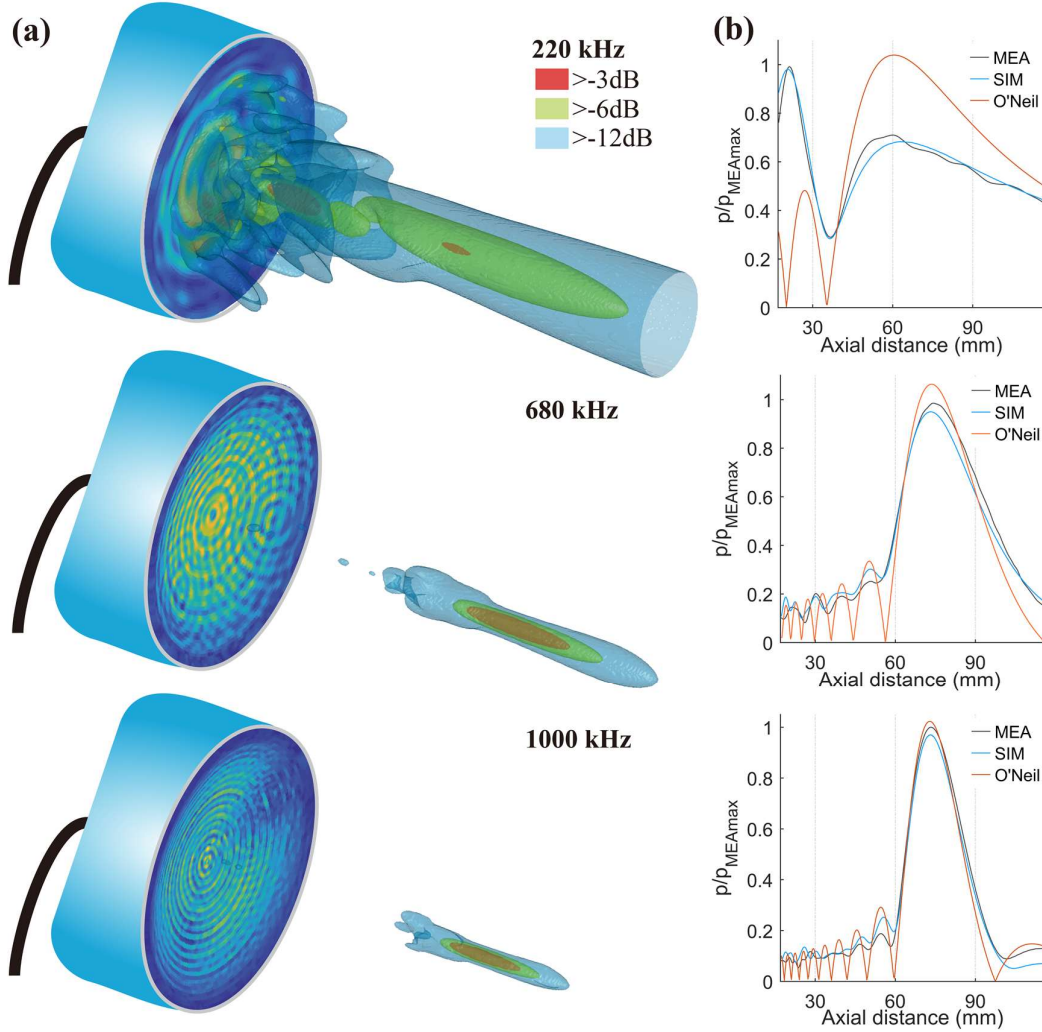


Figure 3-11 (a) Source-surface acoustic pressure reconstructed by backward propagation of the measured holograms, together with the corresponding forward-simulated focal volumes thresholded at -3dB, -6 dB and -12 dB. (b) Comparison of the axial pressure distribution obtained from hydrophone measurement (MEA), simulation (SIM) using the reconstructed source field, and the O'Neil analytical solution. Pressure was normalised to the measured peak pressure.

As shown in Figure 3-11.a, the reconstructed free-field volumes exhibited clear frequency dependence. At 680 kHz and 1000 kHz, acoustic energy was concentrated

near the geometric focus and produced the expected elongated focal volumes. By contrast, at 220 kHz the field above -12 dB was broader and more cylindrical, consistent with more piston-like behaviour. This may reflect a combination of lower-frequency diffraction and transducer-specific deviations from ideal bowl-source behaviour.

Validation of the reconstructed source was performed by comparing forward simulations with the O'Neil solution and free-field hydrophone measurements acquired along the acoustic axis between 18 mm and 118 mm in 0.5 mm increments. As shown in Figure 3-11.b, the reconstructed-source simulation reproduced the hydrophone-measured axial pressure profile more closely compared to the O'Neil solution. Across the three frequencies, the simulated axial peak pressure was approximately 3% to 7% lower than experimental value, and the discrepancy in the location of the spatial peak pressure was limited to approximately 2-3 mm. These deviations indicate that the reconstructed source fields reproduced the essential free-field transducer behaviour with sufficient accuracy to justify their use in subsequent transcranial simulations.

3.3. Discussion

The source characterisation framework described above establishes the physical and experimental basis for the subsequent studies in this thesis. Nevertheless, several practical limitations should be made explicit.

First, the LDV measurements were performed in air rather than under water loading. Because acoustic radiation into water substantially modifies the mechanical boundary condition of the transducer, air-measured displacements cannot be interpreted directly as in-water operating displacements.

Second, the simple plane-wave radiation-force relation is not strictly applicable to the concave focused transducers used in this study, because the emitted field is convergent rather than perfectly plane. Under these conditions, the measured radiation force depends on the component of wave momentum normal to the absorbing target, and a focusing correction should therefore be considered. For a spherically focused bowl

transducer, this correction may be expressed in terms of the beam convergence half-angle θ , the average axial momentum factor can be approximated as $(1 + \cos\theta)/2$. For the transducers used here, this corresponded to approximately 96% of the total acoustic power.

Third, it is worth noting that the voltage was monitored using an oscilloscope configured with a 1 M Ω input impedance. This high-impedance setting minimised loading of the measurement circuit and therefore allowed the terminal voltage to be measured without introducing a 50 Ω termination. This distinction is important because, for a source with a 50 Ω output impedance, the voltage measured under a high-impedance load can approach twice that obtained under matched 50 Ω termination, consistent with a simple Thevenin-equivalent analysis. This was accounted for when reporting voltages and interpreting hydrophone signals.

Fourth, hydrophone sensitivity varied not only with nominal active element diameter but also between individual probes and from day to day. As shown in Table 3-2, representative calibration values at 650 kHz differed substantially across the seven hydrophones used in the project. Daily calibration was therefore necessary to minimise the contribution of sensitivity drift and probe-to-probe variability to the overall measurement uncertainty.

Table 3-2: Comparison of measured hydrophone sensitivities at 650 kHz with manufacturer reference values.

Hydrophone	Sensitivity at 650 kHz, mV/MPa ($\pm$ uncertainty)	Reference value, mV/MPa
HP1, 0.2 mm	33($\pm$ 1.0%)	32
HP2, 0.5 mm	236 ($\pm$ 0.5%)	270
HP3, 0.5 mm	366 ($\pm$ 0.5%)	270
HP4, 1.0 mm	740 ($\pm$ 1.0%)	920
HP5, 1.0 mm	1140 ($\pm$ 3.5%)	920
HP6, 1.0 mm	730 ($\pm$ 3.5%)	920
HP7, 1.0 mm	910 ($\pm$ 5.0%)	920

Fifth, although hydrophone selection is often guided by the quarter-wavelength criterion in IEC 62127, the use of a larger hydrophone may still be practical when improved signal-to-noise ratio is required. Repeated field mappings in the same plane

using hydrophones of different diameters showed negligible differences across the three frequencies tested under the present measurement conditions. Hydrophone-aperture effects were therefore considered small relative to other sources of uncertainty in this study, although this conclusion should not be generalised to all field geometries or higher-frequency measurements. In addition, the hydrophone does not sample the wavefront under perfectly orthogonal incidence across the full aperture. For example, measurements at 680 kHz showed that a 20° tilt reduced the measured peak by approximately 3.5%. Angular sensitivity therefore remains a potential source of underestimation, particularly in highly curved or distorted fields.

Sixth, the source-reconstruction framework represents a substantial improvement over idealised aperture definitions, nevertheless, several factors may affect this reconstructed source distribution. Residual reflections from the hydrophone tip can perturb near-field measurements and contribute to residual near-field error. Peripheral wave information may also be lost when the measured hologram is back-propagated to a virtual detector that does not fully cover the effective radiating surface. Additional uncertainty arises from spatial averaging, finite bandwidth and filtering during signal processing. These effects may lead to systematic underestimation of radiated energy or mild distortion of the reconstructed source field.

Alternative source-modelling approaches commonly avoid explicit source reconstruction either by propagating a measured hologram to a plane and using that plane as a mass source (*calculateMassSourceCW*) (Krokhmal et al., 2025), or by computing an idealised source field directly from transducer geometry (*kWaveArray*) (Yaakub et al., 2023). These approaches are convenient, but they do not explicitly represent the physical boundary behaviour of the piezoceramic surface. This limitation may become important when the sonication train extends over many cycles and the transducer is placed close to a reflecting or scattering interface. This concern is addressed in the Chapter 4 and 5.

Finally, a smaller but practically relevant source of uncertainty arises from rotational freedom during transducer mounting. The reconstructed bowl-surface maps in Figure 3-11 (a-b) reveal an inherent asymmetry. If the transducer is not immobilised about its axis, the measured field cannot be reproduced exactly in simulation.

Overall, this chapter establishes a measurement-informed description of the ultrasound sources used throughout this thesis, spanning their electromechanical behaviour, acoustic power, pressure distribution and complex free-field characteristics. The purpose of this characterisation is not to recover every internal electromechanical detail of the commercial transducers, but to establish a sufficiently controlled and experimentally validated source baseline for subsequent transcranial measurements and modelling. With the incident field characterised, the next chapter investigates how passage through the human skull modifies this baseline.

4. Transcranial characterisation

The purpose of this chapter is to characterise how cranial bone modifies the transmission of focused ultrasound and to identify the skull-related factors that govern intracranial pressure delivery, insertion loss, and beam distortion. The chapter first describes the skull specimens employed, and the CT-based methods used to quantify regional thickness, skull density ratio (SDR), and local curvature. It then presents experimental measurements of transcranial pressure and power transmission, together with analyses of insertion loss, focal distortion, focus expansion, and focus shift across sub-megahertz frequencies. Finally, the influence of transducer-skull angulation and transducer-skull spacing is examined in order to identify practical sources of uncertainty likely to affect tFUS delivery in both experimental and clinical settings.

4.1. Introduction

The preceding chapter established the free-field source characterisation framework used in this thesis. However, for tFUS, the calibrated free-field output of a transducer is only the starting point. Once the beam passes through cranial bone, the field delivered to the intended target may differ substantially from the field measured in water, both in amplitude and in spatial distribution. Some in-vivo studies assume a uniform insertion loss across individuals or apply free-field pressure measurements as a substitute for in-vivo parameters (Fomenko et al., 2020b; Zeng et al., 2024a), despite the fact that this reduction can vary across subjects due to differences in skull properties and frequencies (Pichardo et al., 2011). Such variability may compromise the accuracy of determining optimal ultrasound neuromodulation parameters.

Previous studies have characterised skull transmission primarily using short-pulse measurements and have shown that attenuation increases with both skull thickness and ultrasound frequency (Ammi et al., 2008; Guo et al., 2022a; Kirchner et al., 2023; J. Lee et al., 2020; Pinton et al., 2012a; Riis et al., 2022). However, many neuromodulation protocols employ long bursts containing hundreds of acoustic cycles per pulse, such that the field behaviour is influenced not only by bulk attenuation but also by interference effects associated with skull thickness, skull-transducer spacing, and local skull morphology.

Under these conditions, thickness alone is unlikely to provide a sufficient descriptor of transcranial transmission. (Connor & Hynynen, 2004; F. J. Fry & Barger, 1978; P. J. White et al., 2006a). Clinical MRgFUS treatment for essential tremor (Boutet et al., 2020a) further suggests that SDR, which reflects the contrast between trabecular and cortical layers, is also relevant to transmission efficiency, (D'Souza et al., 2020a). although its role in low-intensity sub-megahertz neuromodulation remains incompletely defined.

Computational studies have likewise shown that accurate prediction of transcranial acoustic fields depends strongly on how skull geometry and material heterogeneity are represented. This motivates a systematic experimental analysis of the relationships among skull thickness, SDR, local curvature, transducer-skull alignment, and the resulting transmitted acoustic field. In this chapter, transcranial field mapping is performed under sub-megahertz, long-burst conditions intended to approximate practical tFUS delivery. By comparing free-field and transcranial measurements, the chapter examines insertion loss in pressure and power, focal distortion, focus expansion, and focus shift, while also identifying practical sources of uncertainty relevant to experimental design and future subject-specific planning.

4.2. Skull specimen

4.2.1. Historical and Thiel-embalmed skulls

The human skull specimens used in this study were obtained from the Centre for Anatomy and Human Identification (CAHID) at University of Dundee. Most specimens were historical skulls, selected because they were more readily available and easier to store under ambient laboratory conditions. Historical specimens were visually inspected from a pool of approximately 200 skulls to capture broad variation in thickness, density, size, and morphology. Although the provenance of the collection is not known, the breadth of anatomical variation suggests that the selected specimens represent a wide range of ages and sexes.

A practical issue specific to historical skulls is dehydration. In vivo, cranial bone contains marrow, vascular structures, and lipid-rich components that occupy the trabecular space. In dehydrated historical skulls, these regions are replaced largely by air. If such specimens are scanned directly in the dry state, as shown in Figure 4-1 (Dehydration), the apparent voxel-averaged density is artificially reduced because the CT value reflects a combined bone-air composition rather than bone with soft-tissue or fluid filling. Because the densities of blood and fatty tissue are approximately 1050

kg/m³ and 900 kg/m³, respectively, water was considered a reasonable replacement medium for rehydration. Four skulls were therefore scanned in both dehydrated and rehydrated conditions. Rehydration increased the mean CT density by 10.9±9.1% relative to the dehydrated state, indicating that specimen hydration has a non-trivial influence on image-derived skull properties. Historical skulls were therefore rehydrated prior to CT imaging. Rehydration was performed by first degassing the specimen under vacuum until no visible air bubbles emerged from cut edges, followed by overnight soaking in water. The skull was removed from water immediately before scanning. Attempts were made to scan specimens while submerged; however, suitable fixation during table translation proved difficult. Consequently, some fluid loss during scanning could not be excluded and should be regarded as a source of experimental uncertainty.

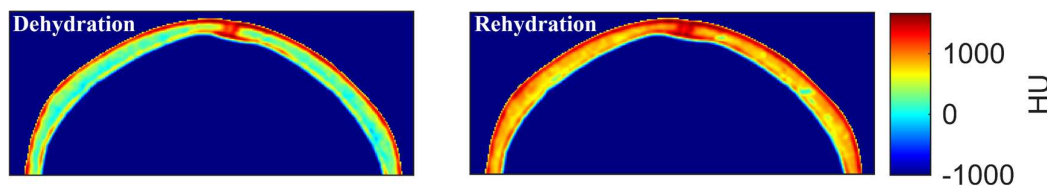


Figure 4-1 CT density distribution of a representative historical skull specimen that scanned in both dehydrated and rehydrated conditions.

Apart from the historical specimens, two Thiel-embalmed calvaria were obtained for comparison. These samples were derived from Thiel soft-embalmed cadavers, most of whom were between 75 and 95 years of age. The specimens retained the elasticity and gross handling characteristics of living tissue, with scalp and dura intact. Although embalming may alter electrical conductivity, viscoelastic behaviour, and stiffness, these changes are not expected to substantially alter ultrasound transmission characteristics (Estermann et al., 2021). All specimens were handled confidentially, and all procedures involving the Thiel material complied with the Anatomy Act 1984 and the Human Tissue (Scotland) Act 2006 (Human Tissue (Scotland) Act 2006).

During the study, historical skulls were stored dry, rehydrated before experiments, and dried again afterwards. Thiel specimens were stored in Thiel fluid, soaked in water before experiments, and returned to Thiel fluid afterwards. Progressive soft-tissue degradation and detachment were observed in the Thiel samples during repeated handling and transport, whereas the historical skulls were mechanically more robust.

Any error introduced by sample changes over time was not quantified and is acknowledged as a limitation.

4.2.2. Skull segments structural characterisation

Eight historical calvaria, two historical half-skulls, and two Thiel-embalmed calvaria were examined. For each half-skull, four regions of interest (RoIs) were selected to approximate clinically relevant brain-target locations (Legon, Ai, et al., 2018b; Sanguinetti et al., 2020b; Yaakub et al., 2023b; Zeng et al., 2024b). For calvarial specimens, these RoIs were mirrored across the midline, giving eight RoIs per calvarium. In total, 88 RoIs were analysed, comprising 72 historical RoIs and 16 Thiel RoIs. The RoI distribution on a representative half-skull is shown in Figure 4-2.(a). Because the RoIs spanned frontal, superior parietal, lateral parietal/temporal, and posterior parietal segments, they sampled a broad range of local anatomical conditions. Treating each RoI as an individual test region increased the effective sampling density while maintaining a manageable number of specimens.

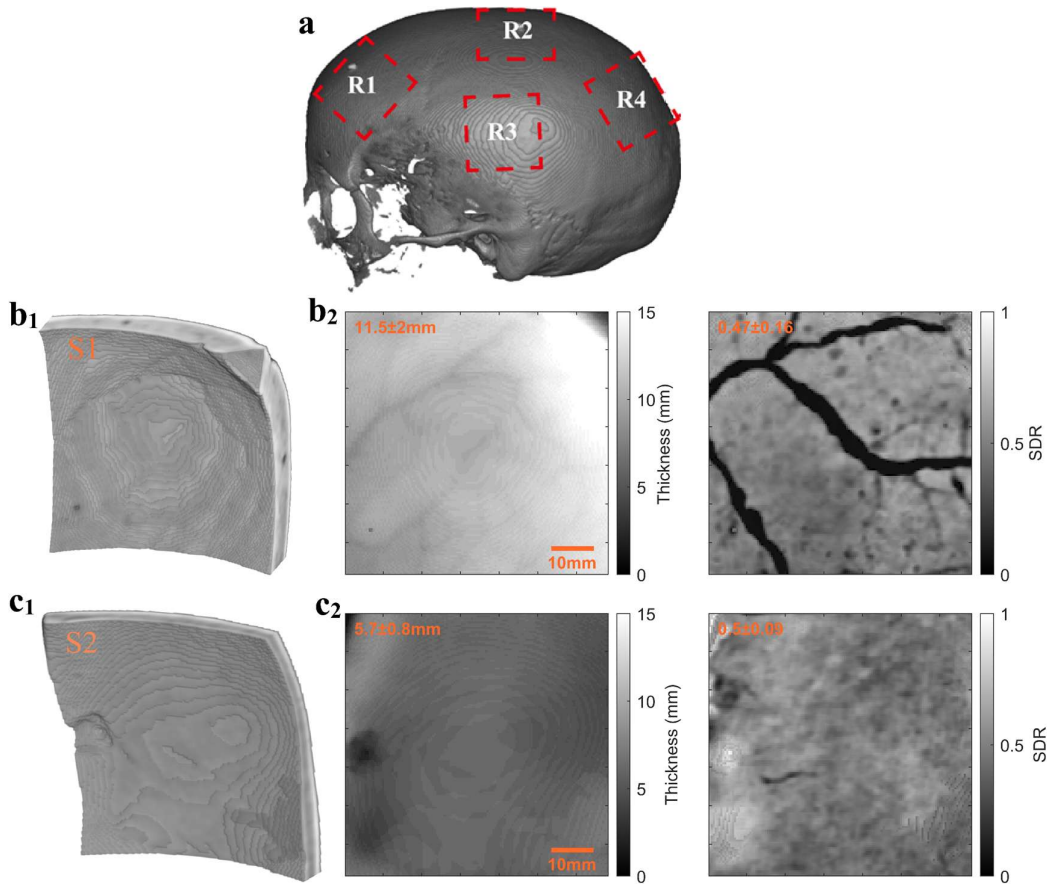


Figure 4-2 A representative skull with RoIs details. Two representative regions were chosen for the following analysis. (a) RoI locations on a half skull; R represent RoIs that are applicable across all skulls. R1 is frontal bone, R2, R3, and R4 are superior parietal, lateral parietal (partial temporal in some samples), and posterior parietal bone, respectively. (b₁) The CT volume of an example RoI (60 mm by 60 mm surface area) labelled throughout as S1; S represent specific sample regions selected from RoIs for illustrative purposes. (b₂) Thickness and SDR map of S1. Average thickness and SDR are 11.5 ± 2.0 mm, and 0.47 ± 0.16 , respectively. The RoC of S1 is approximately 55.1 mm. (c₁, c₂) Example of a RoI labelled as S2. Average thickness, SDR and RoC of are 5.7 ± 0.8 mm, 0.5 ± 0.09 , and 81.6 mm, respectively.

Structural and morphological information was obtained using a clinical CT scanner (GE Revolution EVO CT scanner; with in-plane spacing at 0.44 mm for both axes and slice thickness of 0.625 mm with 0.31 mm interval), thereby preserving direct translational relevance to clinical imaging workflows. Before scanning, RoIs were marked with metallic ball bearings to enable subsequent localization. CT volumes were processed in MATLAB. A surface-casting method, developed as an extension of ray-casting approaches reported previously (Boutet et al., 2020b; D'Souza et al., 2020b) was used to extract the sub-volume corresponding to each RoI. Specifically, an approximately 60 mm by 60 mm square region was selected from the outer skull surface, centred on the

RoI marker. This dimension was selected to correspond to the 60 mm aperture diameter of the transducers, thereby encompassing the skull region intersected by the projected transducer aperture. The marker was then digitally removed, and the inner and outer skull surfaces were defined using a threshold of 300 HU, comparable with previous transcranial ultrasound modelling approaches (Krokhmal et al., 2025). Two representative RoIs, denoted S1 and S2, are shown in Figure 4-2 (b₁ and c₁) and are used throughout the chapter for illustration.

The RoIs were quantified by three bulk characteristics: thickness, SDR and RoC. Effective thickness was calculated using the surface-casting geometry: a line was traced from each outer-surface voxel to the corresponding inner-surface point, and its length was multiplied by the cosine of the predicted insertion angle, defined from the geometry between the local surface point and the transducer focal position. In practice, the geometric focus was assumed to lie 65 mm beneath the RoI centre. This depth resulted from the 75 mm RoC of the transducer and the 10 mm separation between the transducer surface and the skull RoI. The resulting angle-corrected path lengths were averaged to obtain the mean and standard deviation of thickness for each RoI. This procedure was intended to reflect the actual distance travelled by the acoustic wave, rather than an imaging-plane thickness, although it is inherently transducer-specific and may vary with geometry and focal configuration.

SDR was calculated using the same surface-casting framework but without angle correction. To reduce underestimation of trabecular density caused by air re-entry during scanning, a lower threshold of +100 HU was imposed. RoC was estimated by fitting both the inner and outer skull surfaces to best-fit spheres and taking the mean of the two fitted curvatures as the local curvature metric.

The historical RoIs spanned a wide range of properties: thicknesses ranged from 3.1 mm to 13.0 mm, SDR from 0.07 to 0.80 and RoC from 26.8 mm to 113.0 mm. The Thiel RoIs examined were limited to thicknesses between 4.1 mm to 12.8 mm, SDR values between 0.48 and 0.79, and RoC values between 15.4 mm and 114.4 mm. By using different RoIs across the two Thiel samples, we were able to reveal similarities and differences between transmission across historical and embalmed specimens. The averaged thickness, SDR, and RoC for historical RoIs were 6.9 ± 2.1 mm, 0.41 ± 0.21 ,

and 63.3 ± 21.6 mm, respectively, and for Thiel RoIs, the respective values are 7.9 ± 2.4 mm, 0.55 ± 0.14 , and 59.6 ± 28.1 mm. The three characteristics of the grouped four regions data are presented in Table 4-1.

Table 4-1 The mean and standard deviation of skull region-specific parameters over all 88 RoIs tested.

	R1	R2	R3	R4
Thickness (mm)	7.7 ± 2.6	6.4 ± 1.9	5.8 ± 1.9	8.0 ± 2.3
SDR	0.42 ± 0.23	0.46 ± 0.19	0.47 ± 0.21	0.40 ± 0.19
RoC (mm)	54.6 ± 10.5	80.3 ± 17.5	48.5 ± 12.6	51.2 ± 7.7

4.3. Transcranial energy insertion loss

4.3.1. Field mapping

To characterise transcranial transmission, 220 kHz, 650 kHz, and 1000 kHz single-element transducers were driven using approximately 0.5 ms sinusoidal bursts generated by a signal generator (33500B, Keysight, US), the corresponding free-field peak pressures were 0.06 MPa at 220 kHz, 0.25 MPa at 650 kHz, and 0.35 MPa at 1000 kHz, respectively. Acoustic signals were recorded using a 1 mm needle hydrophone (Precision Acoustics, UK). Hydrophone sensitivity calibration and acoustic-power calibration were performed as described in Section 3.2.3.

The waveform was reconstructed using a narrow band-pass filter centred at the transducer frequency, with a gate width of 10 Hz. Signal amplitude was then extracted from 6-25 steady-state waveform cycles by fast Fourier transform, depending on frequency. Free-field reference maps were acquired at 0.5 mm step resolution for each transducer. Lateral scan dimensions were $30 \text{ mm} \times 30 \text{ mm}$ at 220 kHz and $20 \text{ mm} \times 20 \text{ mm}$ at 650 kHz and 1000 kHz. These scan areas were chosen to cover the full width at

half maximum (FWHM) of the free-field beam while remaining sufficiently small for daily calibration and repeated measurements.

Prior to the experiment, each skull sample was degassed as previously described. Following degassing, Thiel skulls were additionally vacuum sealed using an acoustically and visually transparent sealing bag (with tested transmission efficiency > 99%). Sealing prevented Thiel embalming fluid leaking from the sample into the water tank, and also prevented detachment of soft tissues from the skull caused by water ingress. As shown in Figure 4-3, the degassed skull was then clamped on a snake arm that can be locked in any position, and which was fixed on a two-dimensional motorised stage, and positioned between the transducer and hydrophone.

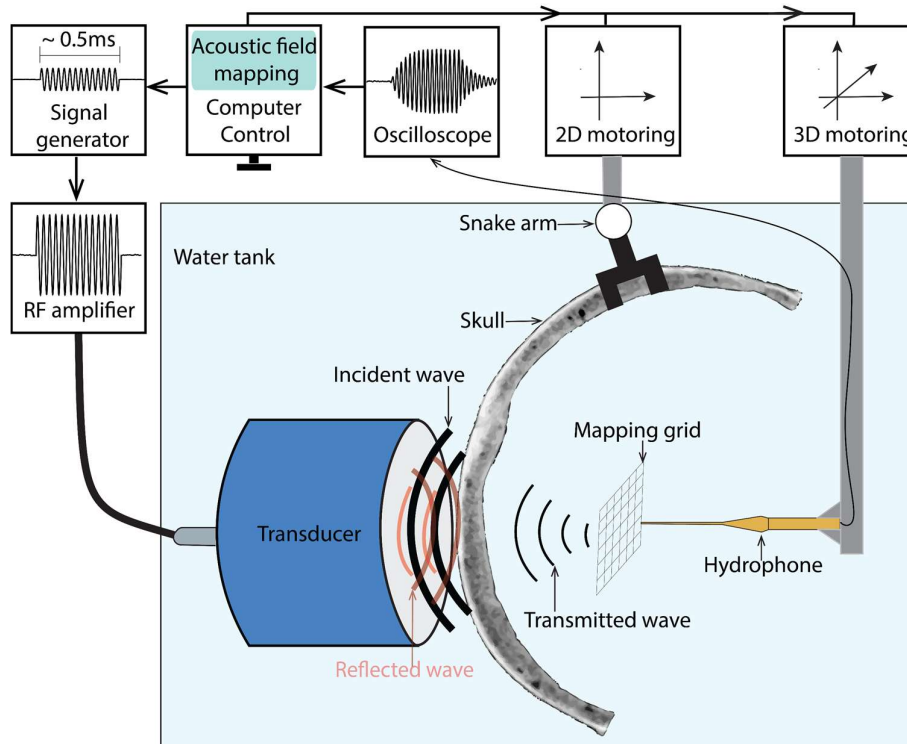


Figure 4-3 Experimental setup of the transcranial acoustic field measurement system.

To investigate the influence of the skull properties on the transmitted pressure and power level, the position of all RoIs under testing were initially aligned to near-normal incidence, maneuvered to approximately 10 mm from the transducer aperture, and then

slightly adjusted to where constructive wave patterns were clearly observed. The approximately 10 mm separation was maintained using a 10 mm-thick hollow spacer placed between the transducer and skull surface, providing a reproducible reference for the transducer–skull distance while allowing water coupling through the central opening. The hydrophone was located at the local maxima along the axial direction, and the acoustic field measurements were taken in the same lateral position as the free-field mapping to capture skull-induced changes in the transmitted acoustic field. The corresponding pressure p at each sampling position and the total acoustic P_{HP} power can be calculated by using equation:

$$P_{HP} = \iint \left(\overline{p(x,y)^2} / \rho c \right) dydx, \quad p = U / M_f, \quad (3.3.1)$$

where U is the signal amplitude, M_f is the hydrophone sensitivity at transducer working frequency, $\overline{p(x,y)}$ is the time averaged acoustic pressure at coordinate (x,y) , ρ and c are the density and sound velocity of water (1000 kg/m³ and 1480 m/s, respectively), $dydx$ is the pixel area (assumed to be the square of the step-resolution). By integrating over the mapping area, we recover the P_{HP} ; the approximate total acoustic power transmitted through that area.

4.3.2. Pressure and power loss

The measured transcranial acoustic pressure and power insertion loss were normalised to free-field values, and the average results are presented in Table 4-2.

Table 4-2 Average transcranial acoustic pressure and power insertion loss from historical and Thiel RoIs.

Historical/ Thiel	220 kHz	650 kHz	1000 kHz
Normalized peak pressure (dB)	-3.6±3.4 -2.9±1.8	-9.3±3.3 -9.4±2.6	-14.8±5.8 -17.0±5.5
Normalized power (dB)	-5.8±5.7 -3.6±1.8	-15.2±6.0 -14.6±2.9	-24.2±9.0 -26.1±8.9

The historical and Thiel results presented in Table 4-2 show average transcranial peak pressure insertion loss. In comparison, another study that tested frequencies of 150 kHz, 500 kHz, and 1000 kHz measured corresponding peak pressure attenuations of 6.6 dB, 8.9 dB, and 18.4 dB, respectively (M. Chen et al., 2023). These attenuation values are extrapolated to be higher than those observed in our study. The average transcranial power insertion loss for historical and Thiel RoIs demonstrated a less than square relationship to the pressure insertion loss.

The results from historical RoIs were aggregated, and fitted to a linear regression model, accompanied by a 95% confidence interval (CI), to demonstrate the correlation between the pressure and power level to the RoI thickness and SDR at the three frequencies. The results from Thiel RoIs (Thiel) were overlaid to the plots, verify the fitness of the results for the two skull types.

Figure 4-4 illustrates the relationship between insertion loss levels of pressure and power and average RoI thickness. At 220 kHz, insertion loss for historical RoIs ranged from -0.6 dB to -8.1 dB, and -1.0 dB to -12.5 dB for peak pressure and power, respectively, relative to free-field values. Thiel RoIs exhibited slightly smaller ranges of -0.6 dB to -6.8 dB, and -1.4 dB to -6.7 dB, respectively. These values appeared to be independent of thickness variations.

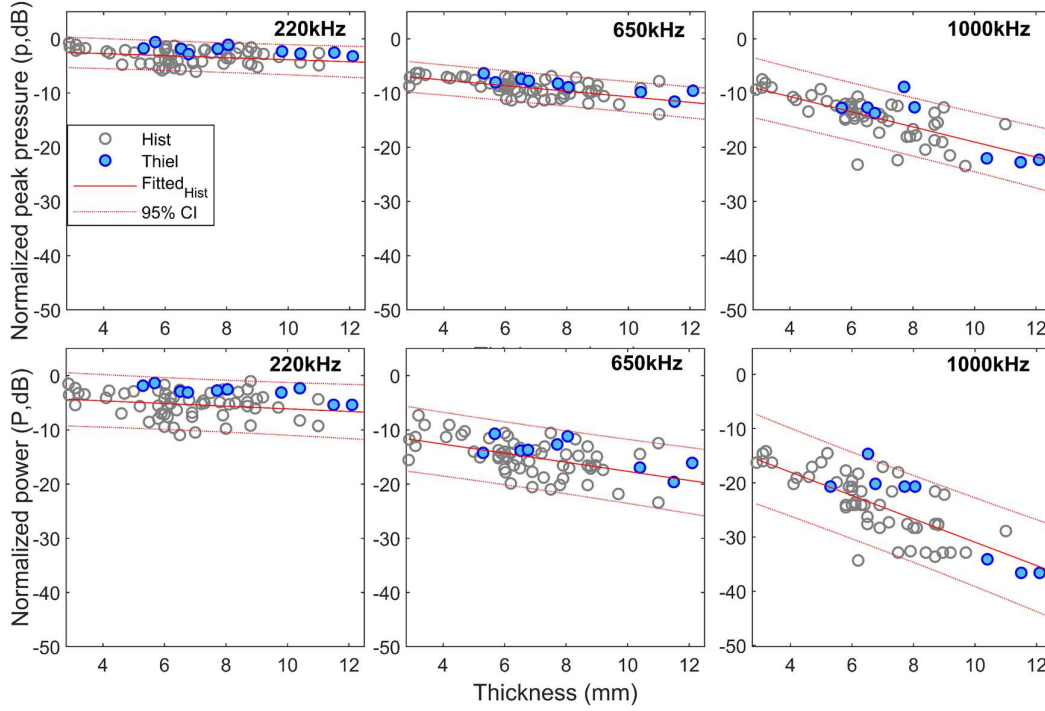


Figure 4-4 Skull thickness versus the normalised transcranial peak pressure level (dB), power level (dB) at three frequencies. The grey circles indicate are historical skull RoIs (Hist), and filled blue circles represent Thiel RoIs (Thiel). The linear fit (plus 95 % confidence interval, CI) to the historical RoI data ($Fitted_{Hist}$) is indicated as shown.

At 650 kHz, Historical RoI insertion loss increased to range from -6.4 dB to -15.5 dB for peak pressure and -7.3 dB to -23.4 dB for power. For Thiel RoIs, the corresponding ranges were -6.4 dB to -16.1 dB, and -10.7 dB to -19.6 dB. The correlation between these insertion loss levels and thickness (TH, mm) were discernible, and fitting revealed the linear regression equations:

$$p_{Hist,650kHz} = -0.5TH - 5.9 \pm 3.3, (R^2 = 0.30)$$

$$P_{Hist,650kHz} = -0.8TH - 9.9 \pm 6.0, (R^2 = 0.24)$$

$$p_{Thiel,650kHz} = -0.6TH - 4.2 \pm 4.3, (R^2 = 0.52)$$

$$P_{Thiel,650kHz} = -0.8TH - 8.6 \pm 4.7, (R^2 = 0.57)$$

Where p and P are pressure and power level, respectively. At the higher frequency of 1000 kHz, insertion loss further increased. Historical RoI values ranged from -7.5 dB to -25.6 dB, and -14.2 dB to -36.7 dB respectively, Thiel RoIs exhibited similar ranges

of -8.9 dB to -25.6 dB, and -14.7 dB to -40.1 dB . The correlations with thickness were more pronounced, as expressed by the equations:

$$p_{Hist,1000kHz} = -1.4TH - 5.4 \pm 5.8, (R^2 = 0.52)$$

$$P_{Hist,1000kHz} = -2.1TH - 10.2 \pm 9.0, (R^2 = 0.50)$$

$$p_{Thiel,1000kHz} = -1.7TH - 3.1 \pm 6.2, (R^2 = 0.77)$$

$$P_{Thiel,1000kHz} = -2.6TH - 4.2 \pm 11.4, (R^2 = 0.69)$$

Notably, the data collected from the Thiel RoIs mostly fell within the confidence intervals for historical RoIs at all three frequencies. Highlighting comparable trends.

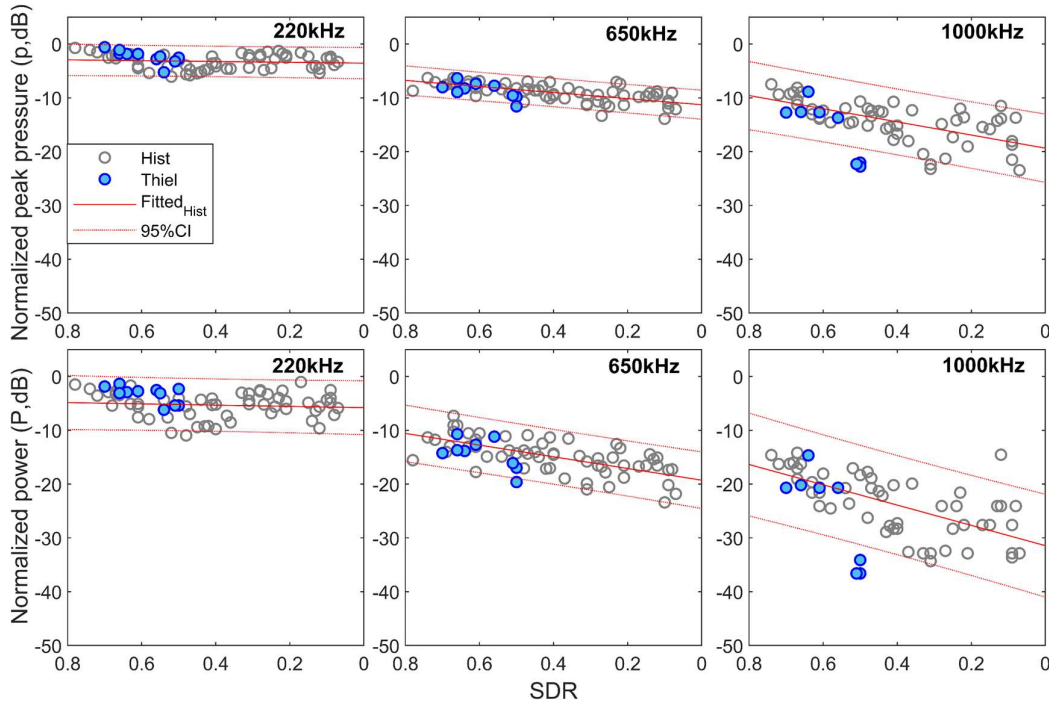


Figure 4-5 Skull SDR versus the normalised transcranial peak pressure level (dB) and power level (dB).

Similar patterns were also observed concerning the correlations between SDR and the insertion loss. As shown in Figure 4-5, at 220 kHz, the insertion loss of pressure and power exhibiting independence from variations in SDR, whereas weak correlations were noted at both 650 kHz and 1000 kHz for Historical RoIs:

$$p_{Hist,650kHz} = 4.4SDR - 11.1 \pm 3.5, (R^2 = 0.22)$$

$$P_{Hist,650kHz} = 9.6SDR - 19.1 \pm 5.5, (R^2 = 0.35)$$

$$p_{Hist,1000kHz} = 12.7SDR - 20.1 \pm 6.6, (R^2 = 0.39)$$

$$P_{Hist,1000kHz} = 19.4SDR - 32.2 \pm 9.9, (R^2 = 0.40)$$

Thiel RoI data mostly aligned with the confidence intervals at 220 kHz, whereas several deviations were apparent at 650 kHz and 1000 kHz. The limited SDR range in Thiel RoIs restricted comprehensive analysis of its correlation with insertion loss.

4.4. Transcranial field distortion

4.4.1. Phase error

The scheme used to analyse the transcranial acoustic field is shown in Figure 4-6, using volume S1 and S2 in Figure 4-2 as examples. In order to compare the transmitted acoustic field across 88 RoIs, the intracranial focal area was defined as the region where the pressure level above the -6 dB (half-maximum) and is shown in Figure 4-6 (a_1 , b_1 and c_1) as the dashed circle on the maps.

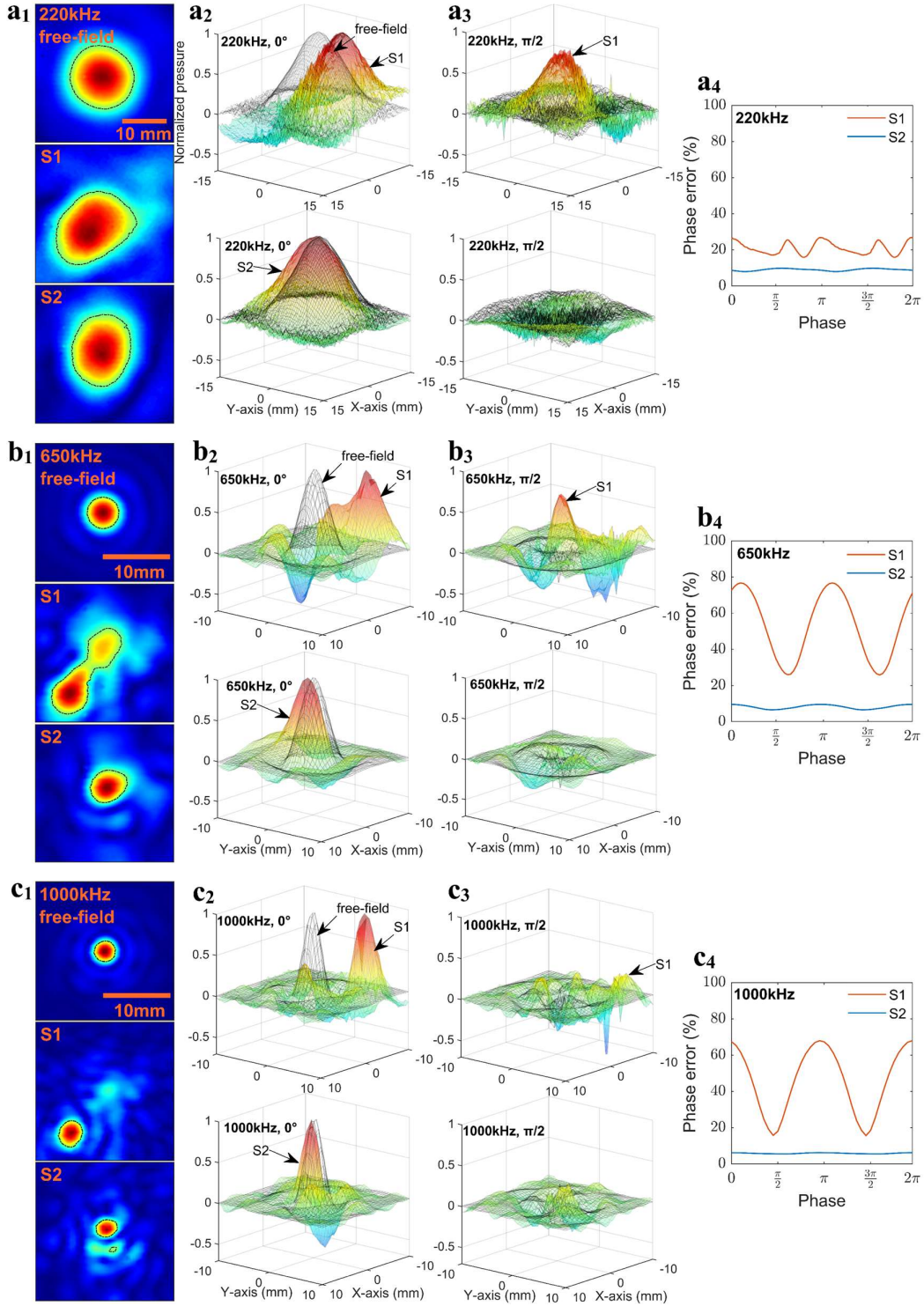


Figure 4-6 Acoustic field aberration analysis for the representative regions S1 and S2 that was described in. Figure 4-2 (a_1 - c_1) Cross-sectional beam area pressure maps at the focal depths of the 220 kHz, 650 kHz and 1000 kHz transducers, produced at the focal depth in both free-field and with the presence of S1 and S2. (a_2 - c_2 , a_3 - c_3) Colour-less grids of three frequencies show a

normalised pressure map at the focal depth in free-field at phase $=0^\circ$ and $\pi/2$, respectively. The composed colour images are those with the presence of S1 and S2. (a4-c4) The full vibration cycle phase errors of the pressure fields of S1 and S2 compared to the reference free-field were recorded at three frequencies.

The aberration of the beam was further assessed by quantifying the normalised pressure-weighted phase errors (pe), which combine information from the pressure and phase maps (Sapozhnikov et al., 2015), by comparing the intracranial focal area and the corresponding area projected onto the reference, using equations:

$$p_{free,inst}(x, y, \psi_{free}) = p_{free}(x, y) \cos(\psi_{free}(x, y)) \quad (4.3.2)$$

$$p_{IC,inst}(x, y, \psi_{IC}) = p_{IC}(x, y) \cos(\psi_{IC}(x, y)) \quad (4.3.3)$$

$$\Delta p(x, y) = \frac{1}{N\psi} \sum_{N\psi} |p_{free,inst}(x, y, \psi_{free}) - p_{IC,inst}(x, y, \psi_{IC})|, \quad \psi \in [0, 2\pi] \quad (4.3.4)$$

$$pe = \frac{1}{N_{IC,focal}} \left(\sum_{N_{IC,focal}} \Delta p(x, y) \right) * 100\%, \quad (x, y) \in IC, focal \quad (4.3.5)$$

Where $p_{free,inst}(x, y, \psi)$ and $p_{IC,inst}(x, y, \psi)$ represent the instantaneous pressures at coordinate (x, y) and phase angle ψ_{free} and $\psi_{free} + \Delta\psi$ in the free-field and intracranial space, respectively. $p_{free}(x, y)$ and $p_{IC}(x, y)$ denote the pressure amplitudes at (x, y) , normalised to the peak values in their respective field. $\Delta\psi(x, y)$ represents the phase shift between the two waves at (x, y) . $N\psi_{free}$ indicates the number of phase samples, spanning from 0° to 2π in increments of 1° , with a small offset applied to the phase angle to prevent division by zero. $\Delta p(x, y)$ is the average pressure-weighted phase difference at (x, y) , and $N_{IC,focal}$ refers to the number of sampled points in the intracranial focal area. The phase error pe , quantifies the average difference of the two fields, specifically focusing to the intracranial focal area. The two fields were synchronized at the moment their peak pressure was reached. This method allows for the comparison of the two wave propagation patterns at the focal zone and visualizes the extent of the wave distortion, providing deeper insights than a simple pressure field comparison.

As two representative RoIs results that illustrated in Figure 4-6 (a₁-c₁), after transmitted through S1, compared to the free-field, the intracranial foci shifted 5.8 mm from the centre of the focal depth at 220 kHz, and 7.8 mm at both 650 kHz and 1000 kHz. The focal area expanded by a factor of 1.1, 3.3, and 1.3 at 220 kHz, 650 kHz, and 1000 kHz, respectively.

As depicted in Figure 4-6 (a₂-c₂ and a₃-c₃), the colourless grids represent the peak (0°, corresponding to the point where peak pressure occurred) and ambient pressure ($\pi/2$) fields in the free-field, and the acoustic fields transmitted through S1 and S2 were represented by the colour grid. The full-cycle pressure vibration error compared to the free-field at each frequency is shown in Figure 4-6 (a₄-c₄). At 220 kHz, phase errors between the two overlapping grids within S1-focal area were 23.3% at peak pressure and 21.7% at ambient pressure. For 650 kHz and 1000 kHz, larger focal shifts resulted in significant deviation in pressure and phase in the S1-focal area with errors of 76.8% and 68.0% at peak pressure, and 26.0% and 15.6% at ambient pressure, respectively. The overall phase errors across a full vibration cycle were 20.9% at 220 kHz, 53.4% at 650 kHz and 46.4% at 1000 kHz.

In comparison, the acoustic field after being transmitted through S2 region exhibited less aberration. The focal shifts were limited to 0.7 mm at 220 kHz and 650 kHz, and 0.5 mm at 1000 kHz. The focal area deviated by less than 5% from the free-field value, with average phase error of 9.0%, 8.1% and 5.5% at three frequencies, respectively.

These representative results suggest that skulls with characteristics similar to the S2 region are ideal for transcranial sonication, allowing straightforward planning without concerns about off-target sonication or inadequate acoustic focusing. In contrast, acoustic field transmitted through RoIs such as S1 revealed severe aberrations, including reduced wave pattern coherence, significant focus shifts, and the absence of meaningful focal point.

To identify bone characteristics beyond average thickness and SDR that contribute to undesired aberrations, all the phase maps were manually inspected, and phase error was fitted to a logistic regression model to define the receiver operating characteristic curve. The determined cut off phase errors were 20.2%, 21.5% and 30.6% for 220 kHz, 650

kHz and 1000 kHz, respectively. Analysis of RoIs exceeding these thresholds revealed a significant factor: excessive variation in thickness across the RoI. Specifically, the RoIs with phase error exceeding the cut-off exhibited thickness deviations of at least 2.0 mm, 1.6 mm and 1.3 mm at 220 kHz, 650 kHz and 1000 kHz, respectively.

The recorded aberration at different phases enables visualization of the coherence of the acoustic beam, although further investigation is required to fully understand the skull properties that cause these aberrations. The average acoustic phase error of the three frequencies acquired from historical and Thiel RoIs are shown in Table 4-3.

Table 4-3 Average transcranial field phase errors of the three frequencies from historical and Thiel RoIs.

Historical/ Thiel	220 kHz	650 kHz	1000 kHz
Phase error (%)	15.5±4.0	16.3±4.8	21.8±10.5
	13.7±7.0	20.7±9.7	28.4±11.4

For context, the phase error in the free-field maps was in the range of 5%-8%, such variations might stem from measurement inconsistency due to finite sampling resolution and slight repositioning mismatch, however, empirically, errors below the cut-off values of 20.2%, 21.5% and 30.6% at the respective frequencies typically exhibited insignificant deviation from the free-field. The cut-off error tolerance increased with frequency due to the more concentrated focal area and the greater phase difference between two adjacent points. Consequently, at higher frequencies, even small deviations could result in significant phase errors.

4.4.2. Focus expansion

In investigating the effect of skull curvature on bending ultrasound wave transmission, and its influences the sharpness of the focus, Figure 4-7 shows that the focal area slightly expanding along increasing skull curvature at three frequencies.

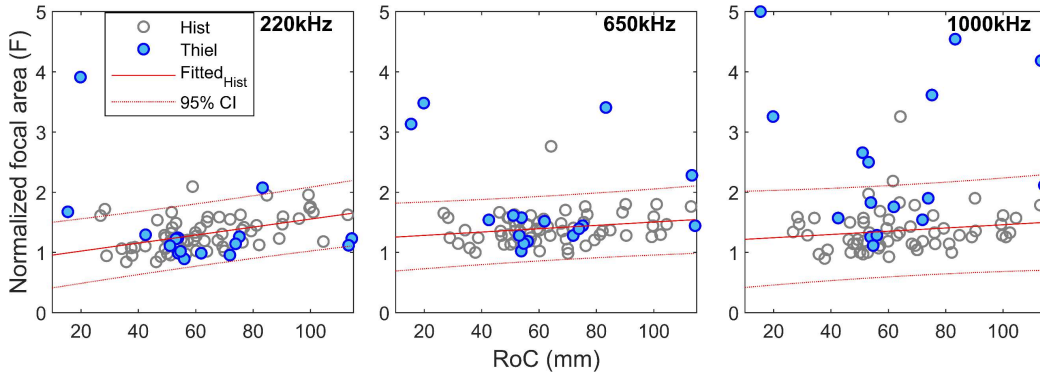


Figure 4-7 Skull RoI RoC versus the normalised focal area (F) at three frequencies. The focal areas were normalised to the free-field reference- a larger ratio indicates a blurred focus, and a smaller ratio indicates a sharper focus.

At 220 kHz, the focal area for historical RoIs varying between a factor of 0.8 to 2.1 relative to the free-field, demonstrating a weak correlation with the skull curvature (RoC, mm):

$$F_{Hist,220kHz} = 6.6 * 10^{-3} RoC + 0.9 \pm 0.5, (R^2 = 0.22)$$

Where F is the intracranial focal area normalised to free-field. However, the focal area varied by a factor of 0.9 to 2.7 for 650 kHz and by 0.9 to 3.3 for 1000 kHz, with no discernible correlation to the skull curvature. Although most data from the historical RoIs at 220 kHz align with confidence intervals, deviations were more pronounced at higher frequencies, particularly for Thiel RoIs. This suggests that factors other than curvature also dominate at these frequencies.

As shown in Table 4-4, spatial spreading of the acoustic energy is evident, as on average the focal area expanded at the three frequencies, indicating that, in practice, a sonication may affect more brain tissue than intended in order to produce the same level of pressure at the brain target, because compensating for skull-induced attenuation may require increased source output, while focal broadening and aberration can simultaneously expose a larger volume of surrounding tissue to appreciable acoustic pressure.

Table 4-4 Average transcranial field expansion ratio of the three frequencies from historical and Thiel RoIs.

Historical/ Thiel	220 kHz	650 kHz	1000 kHz
Normalized field expansion	1.3±0.3 1.4±0.7	1.4±0.3 1.8±0.8	1.4±0.4 2.7±1.9

4.4.3. Focus shift

The average focus shift compared to the free-field were recorded and is shown in Table 4-5. Notably, the average shifts at 220 kHz were larger than 650 kHz and 1000 kHz, despite lower average phase error. This is partly attributable to the longer wavelength and broader focal region at 220 kHz, for which small changes in the pressure distribution can displace the location of the peak without substantially disrupting phase coherence. The observed shifts generally remained within the FWHM of the free-field focus and therefore represented relatively limited distortion of the overall focal region.

Table 4-5 Average transcranial focus shift of the three frequencies from historical and Thiel RoIs

Historical/ Thiel	220 kHz	650 kHz	1000 kHz
Focus shift (mm)	2.3±1.9 2.0±1.5	1.1±1.1 0.8±1.1	0.9±1.2 1.3±1.0

4.5. Transcranial energy uncertainties

4.5.1. Angulation effect

Using the 650 kHz transducer and a skull piece (thickness of 6.2 ± 0.8 mm, SDR of 0.54 ± 0.18 , and RoC of 56.1 mm), the effect of transducer-skull angulation was also

investigated. The relative angle was adjusted from 0° to 15° , then to 30° , and the resulting field aberration was tested and is illustrated in Figure 4-8.

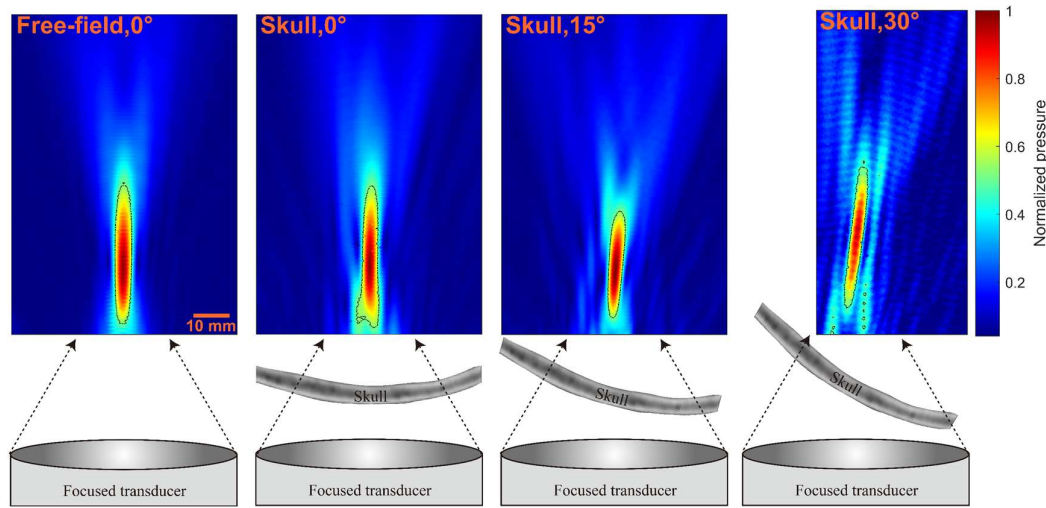


Figure 4-8 Acoustic field visualisation of the 650 kHz transducer beam profile in free-field and with the presence of a representative skull (thickness of 6.2 ± 0.8 mm, SDR of 0.54 ± 0.18 , and RoC of 56.1 mm) at approximate angulation of 0° , 15° and 30° to the right side of the transducer aperture. Pressure is normalised to its maximum value, the area enclosed by the dashed circle is where the pressure is above -6 dB of the peak pressure. The manoeuvring of the hydrophone was blocked by the cranial bone at a 30° alignment, resulting in a limited field of view.

In Figure 4-8, the maximum beam depth and width above -6 dB is 37.0 mm and 4.0 mm in free-field, this is the same as when the skull piece was aligned normal to the beam. The depth and width are reduced to 28.0 mm and 3.5 mm at a relative angulation of 15° and 36.5 mm and 3.0 mm at a relative angulation of 30° . The propagation direction appeared to be shifted by angulation of the RoI by 5° for a 15° angulation and by 7° for a 30° angulation. The peak intracranial pressure in the acoustic field with the skull piece at 0° and 15° were reduced to -9.8 dB and -10.8 dB of the free-field, respectively, and located close to the geometrical focus. Increasing the incident angle to 30° led to a peak pressure drop of -28.0 dB and shifted the focus 8.0 mm from the geometrical focus

These results indicate that it is important to keep the RoI near normal to the transducer aperture, to avoid excessive phase aberration or excitation of shear modes. At oblique incidence, the longitudinal wave incident on the skull can undergo mode conversion at the fluid–bone interface, with part of the acoustic energy propagating as a shear wave within the skull. As shear waves have different propagation velocities and attenuation

characteristics from longitudinal waves, this mode conversion may contribute to the increased transmission loss and altered propagation direction observed at larger incident angles. However, the longitudinal and shear components were not separately measured in this study, and their individual contributions therefore cannot be quantified. Additionally, a slight tilt of the transducer appears acceptable, offering a larger acoustic window for tFUS clinical applications, as it can, in some cases, have less impact on the acoustic field than transmitting through skull regions with undesirable morphology.

4.5.2. Wave superposition effect

During early testing of longer-duration sonication, a wave superposition effect started to be noticeable across the RoIs. To investigate the impact of transducer-skull spacing on the transmitted acoustic pressure level, using a single RoI, the gap between the skull and the transducer aperture was increased from 10 mm to 50 mm, in increments of 0.2 mm, and the pressure at the focal point was recorded.

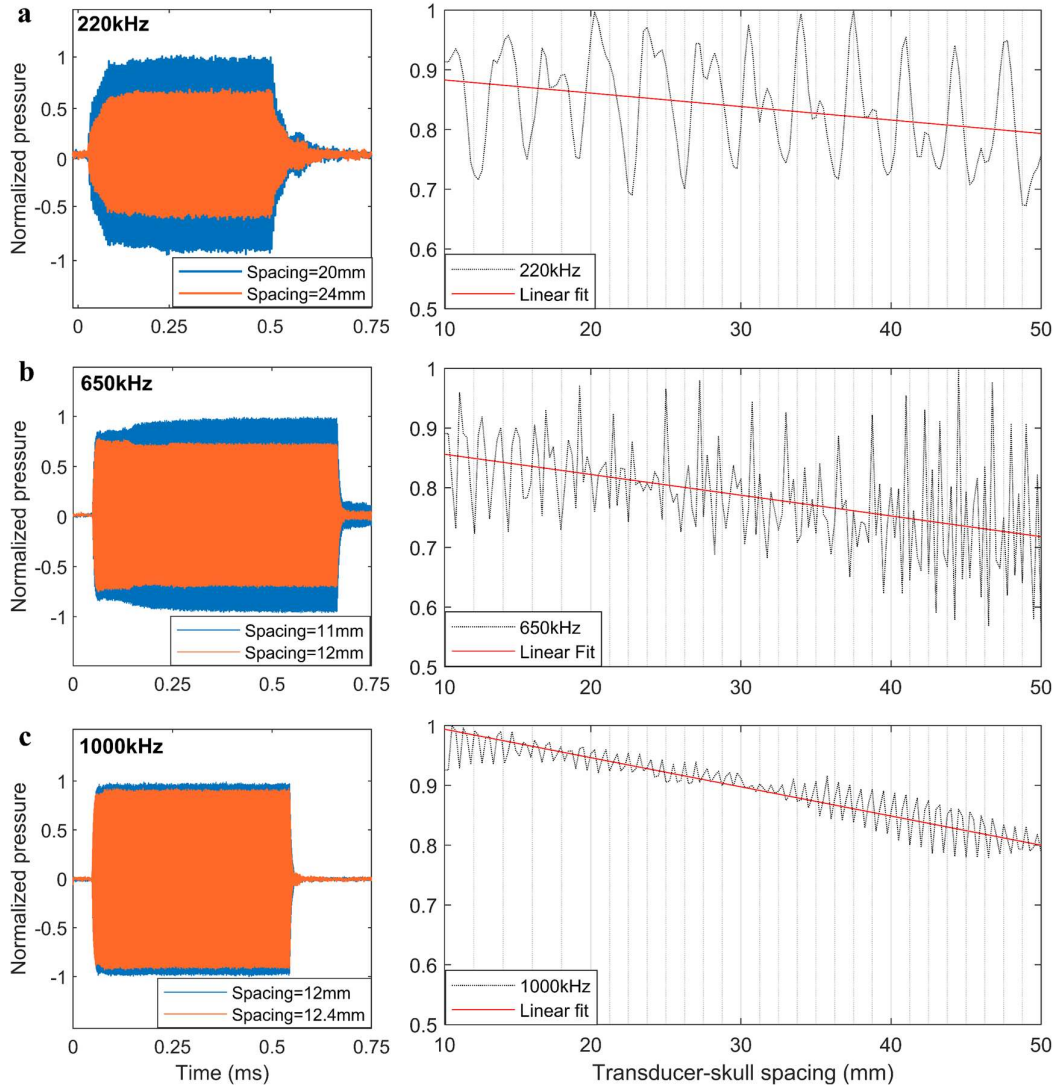


Figure 4-9 Pressure variation at the intracranial focus along with the increase of the transducer and representative skull (thickness of 3.2 ± 0.8 mm, SDR of 0.65 ± 0.25 and RoC of 70.0 mm) spacing at three frequencies and its waveform. (a-c) Waveform amplitude development at spacing approximately of 20 mm and 24 mm, 11 mm and 12 mm, 12 mm and 12.4 mm, and the pressure variation from 10 mm to 50 mm spacing at 220 kHz, 650 kHz, and 1000 kHz, respectively. The linear fitted red line showed the overall decreasing trend of the pressure along the spacing.

As shown in Figure 4-9, significant interference effects are evident at 220 kHz and 650 kHz. The recorded intracranial peak pressure exhibited an oscillation pattern as the transducer-skull spacing increased from 10 to 50 mm. The pressure difference between the waveforms exhibiting constructive and destructive interference is approximately 30% to 40% at 220 kHz and 650 kHz. This effect was mitigated at 1000 kHz, with much lower pressure variation of roughly 10%. The linear fitted results demonstrated a

decreasing trend as spacing increased. Specifically, the average pressure reduction for each 10 mm increment was observed to be approximately 2.0%, 3.5%, and 5.0% at 220 kHz, 650 kHz, and 1000 kHz, respectively.

This phenomenon is prominent across the sub-MHz frequency domain and is contingent upon the specific skull morphology. To mitigate standing wave effects, options could include chirped waveforms, random phase-shifting, or using phased-array transducers (Tang & Clement, 2010; S. Yoo et al., 2022b), however, none of these are universal solutions for narrow-bandwidth, single-element transducers that most studies use. Furthermore, within an intact skull, reflections from the inner cranial surfaces can interfere with the transmitted wave, producing spatial pressure maxima and minima away from the intended focus. Our experiment did not utilise intact skulls, therefore we provide no quantification of standing wave effects within the intracranial domain.

Our findings strongly indicate that wave superposition effects must be accounted for during FUS delivery to allow for FUS dose at the focal point to be accurately estimated. We have not measured the temperature throughout the experiment, as pressure levels are considerably lower than the safety level proposed by International Transcranial Ultrasonic Stimulation Safety and Standards (ITRUSST).

4.6. Discussion

To accurately capture acoustic field variations during sonication, limits were set on the temporal and spatial scope of data acquisition. The mapped transverse planes extended across at least twice the FWHM of the free-field focus. This provided adequate data collection in instances of focus shift or expansion, but also allowed field mapping to be completed within an hour. Axial scans were performed to visually inspect the severity of beam distortion caused by variations in transducer-skull angulation. Due to the time-intensive nature of this procedure, axial mapping was not applied to each RoIs. The pulse duration was set to approximately 0.5 ms. Though typical pulses delivered in

human studies may extend beyond this range, (Fomenko et al., 2020b; Zeng et al., 2024a) 0.5 ms is long enough for the characteristics of continuous-wave sonication to develop.

This experiment primarily focused on examining general transcranial FUS performance, rather than performance when engaging with a specific brain region. Multiple locations were tested on each calvarium, including the frontal, parietal and partly included temporal bone. As illustrated in Figure 4-2, four regions, separated by the groove for superior sagittal sinus and coronal sutures were selected.

The R1 region, situated within the frontal bone, is generally characterised by their thickness (7.7 ± 2.6 mm), but it also features a smooth outer surface and comparable RoC (54.6 ± 10.5 mm) to the transducer. In practice, waves propagating through this region exhibited minimal focus shift and phase aberration. However, two RoIs within the R1 region featuring a bulging inner table, with a curved outward inner surface, resulted in an erroneous calculated inward curvature (RoC of 15.4 mm and 19.8 mm, thickness deviation of 2.3 mm and 2.0 mm). The transmitted sound beam exhibited significant phase aberration at all three frequencies resulting in average focal area increased by a factor of 2.6, 3.3, and 4.1, with corresponding average phase errors of 25.4%, 38.9% and 44.4% for 220 kHz, 650 kHz, and 1000 kHz, respectively. An RoI with a thickness deviation of 1.4 mm caused the acoustic field to develop a sub-focus at 1000 kHz, enlarging the focal area to 2.9 times its size in the free-field, and causing a phase error of 39.0%. In contrast, at 220 kHz and 650 kHz, the field remained largely undistorted, with phase errors of 14.5% and 13.1%, respectively.

The R2 region is located on the superior parietal bone, which is thin (6.4 ± 1.9 mm) compared to the R1, frontal bone region, and R4, posterior parietal bone region (8.0 ± 2.3 mm). However, the RoC (80.3 ± 17.5 mm) is generally larger than in other regions, and larger than the transducer curvature. This caused significant refraction of the sound beam, acting as a diffuser and resulting in a relative lowered peak pressure and broader coverage volume, particularly evident at 220 kHz. An RoI with thickness deviation of 1.6 mm, causing phase error of 9.3% at 220 kHz, However, displaying more pronounced phase error of 34.0% and 45.0% at 650 kHz and 1000 kHz respectively.

The R3 region is positioned on the lateral parietal and temporal bone and temporal bone. Although the structures in this region are on average the thinnest (5.8 ± 1.9 mm), the irregularities of the surfaces and strong curvature (48.5 ± 12.6 mm) contributed to greater phase aberration, reducing the transmission efficiency. One RoI involving the squamosal suture, with a thickness standard deviation of 2.1 mm, the transmitted pressure was dropped 44% to 56% for three frequencies compared to an adjacent location excluding the squamosal suture with the same mean thickness and a thickness standard deviation is 0.6 mm.

The R4 region, located at the posterior of the parietal bone, had RoIs with a relatively smaller RoC (51.2 ± 7.7 mm) compared to the RoC of the transducers, this often results in which acting as a confocal lens for the sound beam. This configuration reduced acoustic spreading, enabling precise and even tighter focus field control. Results from transmission through this region were overall satisfactory, except for one RoI with a thickness deviation of 1.5 mm, which caused significant distortion in the 1000 kHz field, preventing the formation of a meaningful focus.

When the transducer-skull spacing was maintained between 10 to 20 mm, approximately 0.2 ms for 220 kHz and 650 kHz, and 0.1 ms for 1000 kHz were required for waveform stabilization. For application scenarios using such short time pulses, determining precise insertion loss is challenging, as wave interactions within the skull thickness and between transducer-skull spacing are still stabilizing during this period. The absence of tone burst waveform with a few cycles limited the analysis to system insertion loss, preventing direct comparisons with skull attenuation. Additionally, the close spacing between the skull and transducer complicated distinguishing wave interactions occurring at the transducer-skull spacing from those within the skull thickness.

Finally, efforts were undertaken to integrate brain phantoms with the Thiel skull for the comprehensive transmission efficiency assessment. Polyvinyl alcohol-based brain phantoms were prepared by following the recommended procedure (Taghizadeh et al., 2018). The prepared phantom has a sound speed of 1520 m/s and an attenuation coefficient of 0.62 ± 0.11 dB/cm/MHz in the sub-MHz frequency, these parameters are comparable to the reported human brain parameters, which are 1530 m/s and 0.58 ± 0.35

dB/cm/MHz^{1.3}.(Duck, 2013) However, we were unable to overcome challenges in sealing the skull plus phantom system in the timeframe of this experiment.

5. tFUS analytical solution

The preceding chapter showed experimentally that transcranial tFUS delivery cannot be predicted from free-field source output or mean skull thickness alone. Insertion loss, focal distortion and phase error were shaped by a combination of skull thickness, SDR, local morphology, insertion angle and transducer-skull spacing. These findings motivate a predictive framework that can translate CT-derived skull information and source geometry into an estimate of intracranial pressure delivery. The aim of this chapter is therefore to develop a CT-informed analytical model for rapid estimation of transcranial insertion loss under sub-megahertz tFUS conditions. It introduces a target-based ray-tracing framework for skull-characteristic mapping and a six-layer analytical transmission model that preserves both intra-skull reflections and spacing-dependent interference between the transducer and the skull, and its performance is validated against k-Wave simulation and hydrophone measurements in ex-vivo skull specimens.

5.1. Introduction

The experimental results in Chapter 4 demonstrated that transcranial acoustic delivery is governed by more than bulk skull attenuation. Although insertion loss increased with frequency and was partly related to thickness and SDR, the transmitted field was also affected by local thickness variation, curvature, insertion angle and transducer-skull spacing. These factors are especially important for long-burst tFUS protocols, where interference can develop both within the skull and in the water path between the source and skull. A rapid model is therefore needed to estimate whether a planned sonication is likely to deliver the intended pressure at the target, and how this delivery changes with frequency, skull location and transducer placement.

Existing analytical and ray-tracing approaches, including Kranion, Exablate-related planning methods and other in-house frameworks, have been developed primarily for phase correction and element selection in array-based systems (Clement & Hynynen, 2002; Lu et al., 2022). These methods generally extract target-specific skull thickness, insertion angle and, in some cases, SDR, and several have shown useful agreement with hydrophone measurements (Jin et al., 2020) or full-wave simulations (Bancel et al., 2021a). However, many simplified models use spatially averaged skull properties, neglect multiple internal reflections between skull layers, or omit the physical transducer and the water path between the transducer and skull. These omissions become important under long-burst sonication, where constructive and destructive interference can influence transmitted pressure.

For tFUS neuromodulation, the relevant objective is therefore not only to estimate attenuation, but to predict frequency-dependent insertion loss under realistic working conditions. Recent analytical work has demonstrated the feasibility of CT-informed transmission mapping (Attali et al., 2023), but direct ex-vivo validation under transducer-present conditions remains limited.

The present chapter addresses this need by developing a CT-informed analytical model that integrates skull thickness, SDR, insertion angle, local layered morphology and transducer-skull spacing within a single framework. The model is designed to preserve the dominant wave interactions governing transcranial transmission while remaining computationally tractable for practical planning. Its performance is benchmarked against k-Wave simulations and hydrophone measurements across ex-vivo skull specimens and sub-megahertz frequencies.

5.2. Derivation of skull properties

The experimental findings in Chapter 4 showed that transmission loss and field distortion are shaped by both bulk skull properties and local geometry. To incorporate these factors into an analytical model, acoustic properties must be assigned along each target-specific skull path. This section defines how longitudinal sound speed, mass density, effective attenuation and three-layer skull morphology were estimated from CT and supporting time-of-flight measurement.

5.2.1. Longitudinal sound speed

The longitudinal speed of sound in skull bone governs phase accumulation and therefore strongly influences interference patterns and insertion loss. However, reported values vary substantially with specimen condition and measurement method (Marsac et al., 2017b), and direct layer-specific measurement was not practical in the present study. Instead, bulk longitudinal sound speed was estimated experimentally using a time-of-flight method.

A 1000 kHz plane transducer (Precision Acoustics, UK; $D = 23$ mm) was positioned approximately 10 mm from the outer skull surface, and a 1 mm needle hydrophone was placed 80 mm away along the acoustic axis. As depicted in Figure 5-1.a, two-cycle bursts were transmitted in both free-field water and through the skull sample. The time advance of the peak relative to the free-field condition was then used to estimate the effective longitudinal sound speed of the specimen

$$c_{bone} = \frac{c_{water}}{1 - \frac{c_{water}\Delta t}{d_{bone}}} \quad (5.2.1)$$

Where Δt is the time advance relative to water, d_{bone} is the RoI thickness, c_{water} is the sound speed in water. Each sample was measured five times and the results averaged. Because the speed of sound in water varies slightly with temperature, it was measured before each experiment rather than assumed constant. Across 25 measurements, the water sound speed was 1480.7 ± 14.3 m/s, close to the nominal value of 1482.4 m/s at 20 °C.

Bulk longitudinal sound speed was measured at 83 locations in 18 historical calvaria and 10 locations in two Thiel calvaria. As shown in Figure 5-1.b and c, moderate linear relationships were observed between CT-derived bulk density and measured sound speed in both specimen groups

$$c_{Hist} = 0.7HU + 1730 \pm 110, \quad R^2 = 0.69; \quad (5.2.2)$$

$$c_{Thiel} = 1.5HU + 774 \pm 107, \quad R^2 = 0.68. \quad (5.2.3)$$

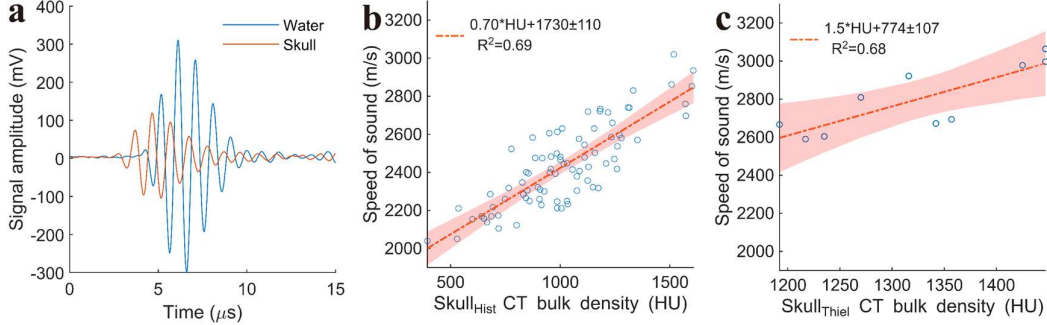


Figure 5-1 (a) Representative time-advance measured with a skull sample compared with in free-field water condition. (b) and (c) The speed of sound measured from 83 locations from 18 Historical skulls and 10 locations from 2 Thiel skulls, respectively. The correlations between the CT density and sound speed are shown as the red dashed lines.

The historical skulls showed substantial spread in sound speed even at similar bulk density, with deviations of up to approximately 400 m/s. The Thiel results showed less scatter, although the smaller sample size may bias the fitted relation. These results indicate that CT-derived density can provide a useful first-order estimate of skull sound speed, but does not uniquely determine it.

5.2.2. Mass density and attenuation coefficient

In contrast to sound speed, mass density can be inferred from CT data with greater confidence. The conversion used here is the *Hounsfield2density* implementation in k-Wave for bone, and is based on established CT-density calibration formulations (Schneider et al., 1996) and is given as

$$\rho_{bone} = 0.66HU + 1011. \quad (5.2.4)$$

The attenuation coefficient used in the analytical model was intended to represent the effective loss due to absorption, scattering, and related propagation losses through the skull. Guided by the insertion-loss measurements reported in Section 4.3, attenuation was parameterised empirically as a function of thickness, SDR, and frequency:

$$a_{TH} = 4 \cdot F^2 \cdot (dBcm^{-1}), \quad a_{TH} \in [a_{cortical}, a_{diploe}]; \quad (5.2.5)$$

$$a_{SDR} = 17(1 - SDR) \cdot F^2 (dBcm^{-1}), \quad a_{SDR} \in [a_{diploe}]; \quad (5.2.6)$$

where F is the frequency per 1000 kHz. These expressions assume a constant absorption ratio a_{TH} through the bone thickness, modified by a density-contrast factor a_{SDR} intended to capture additional loss associated with heterogeneous structure. Because the calibration was obtained from measurements up to 1000 kHz, the resulting attenuation model should be regarded as valid only within that frequency range.

5.2.3. Dynamic three-layer segmentation

The skull was approximated as a three-layer structure consisting of the cancellous diploë between the cortical outer and inner tables. Although actual cranial architecture is spatially heterogeneous, this approximation retains the dominant layered morphology while remaining analytically tractable. A representative example is shown in Figure 5-2.a and b.

An in-house algorithm was developed to segment the skull from the surrounding medium and to identify local layer boundaries dynamically from the CT density profile. CT images exhibit blur across strong contrast interfaces, and skull boundaries may therefore span several voxels, particularly at air-bone transitions where contrast can

exceed 2000 HU (see Figure 5-2.c). Thresholding is therefore required to define the physical interface.

As depicted in Figure 5-2.d, for segmentation of the skull from the environment, the outer and inner surfaces were both defined at 300 HU. The further rise in HU from the outer surface to the first density peak, and the corresponding fall from the second peak to the inner surface, were used to recover representative peak values and thereby compensate partly for air-bone averaging.

To segment the diploë from the cortical layers, thresholds were set initially to approximately 70% and 80% of the first and second peaks, respectively, and adjusted dynamically in lower-contrast regions to preserve a three-layer structure. Where only one density peak was present and the layer structure could not be resolved reliably, the thickness was divided equally among the three layers.

The resulting segmented skull structure, together with the assigned density, sound-speed, and attenuation maps, is illustrated in Figure 5-2.e and f. This dynamic segmentation procedure provides a voxel-wise local three-layer approximation without requiring manual definition of layer boundaries.

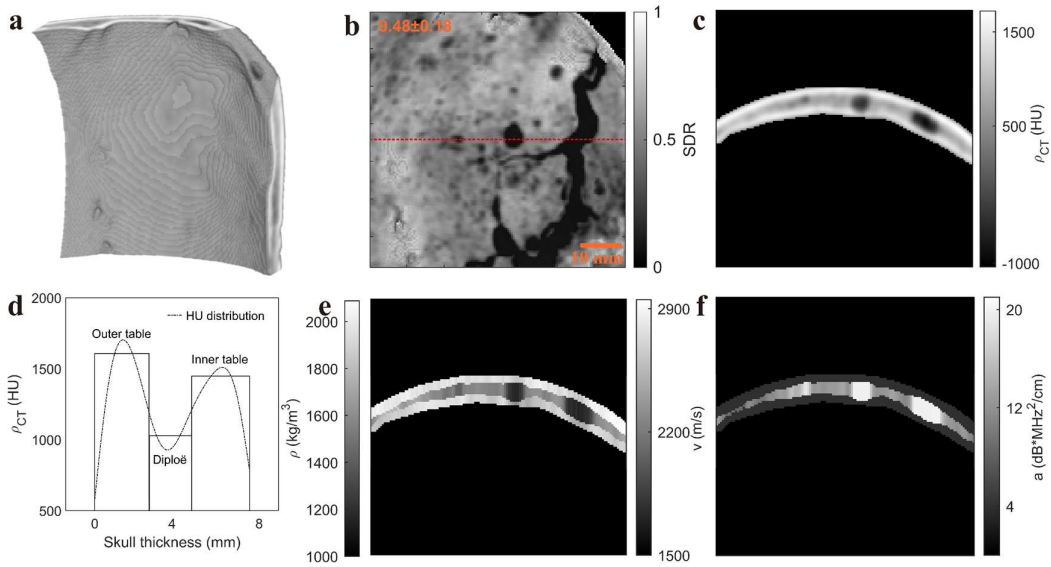


Figure 5-2 (a): a representative historical skull segment. (b) SDR map of the skull segment, and (c) the cross-sectional CT image of the skull segment at the red dashed line. (d) Spline represents the HU distribution along the one voxel-width skull thickness. The bars represent

the segmented layers thickness and its assigned HU value. (e, f) Reconstructed three-layer skull density and sound speed, and attenuation coefficient maps.

5.2.4. Target-based ray tracing and skull-characteristic mapping

The local surface-casting approach described in Section 4.2.2 is effective for small skull segments, but becomes less practical for target-based whole-skull analysis because the skull is globally curved and the local casting window cannot be assumed to remain parallel to the surface over larger regions. A direct ray-tracing pipeline was therefore developed.

A hemi-sected historical skull was used to demonstrate the workflow. As shown in Figure 5-3.a and b, the CT volume was first segmented from the surrounding medium, and a target was placed at the intended brain location, here approximating the thalamus. A total of 800,000 uniformly distributed rays were projected from outside the skull toward the target; for calvaria samples, this number was reduced to 400,000. The ray density exceeded the number of surface voxels, and duplicate paths intersecting the same skull trajectory were removed. Each valid ray was then evaluated using the segmentation and acoustic-property framework described above.

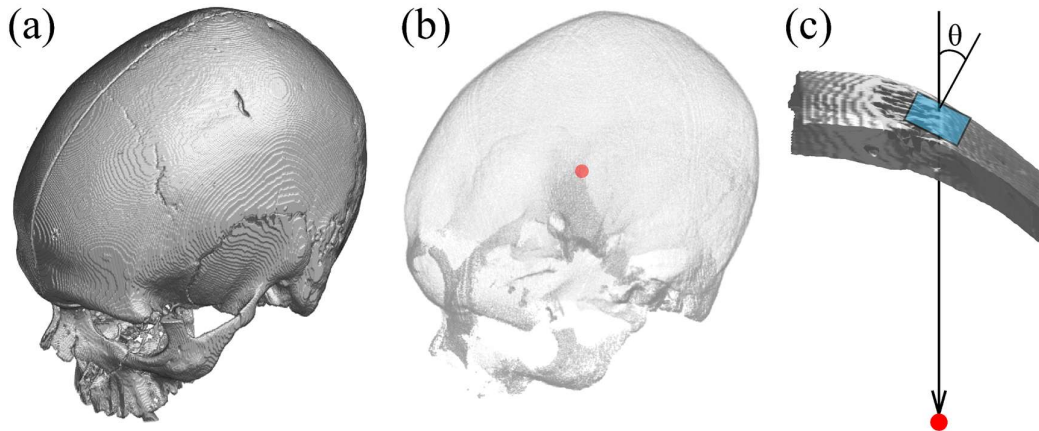


Figure 5-3 (a) A representative CT hemi-sected skull volume. (b) Skull surface map. Red dot represents an approximated brain target at thalamic region. (c) A representative ultrasound insertion line from the seed to the brain target. Blue shade represents the fitted flat outer surface and its angulation relative to the insertion line. Inner surface angulation was not presented but also considered.

The geometric accuracy of the ray-tracing pipeline was assessed by comparing CT-derived thickness with calliper measurements. Three historical calvaria, labelled S1-S3,

were measured manually at 136, 177, and 177 positions, respectively, spaced at 10 mm intervals across the skull surface. The ray-tracing method produced 246,229, 308,450, and 305,528 thickness estimates for these specimens. As shown in Table 5-1, the CT analysis reproduced thickness values with negligible mean error relative to the calliper measurements, supporting the accuracy of both the ray-intersection procedure and the 300 HU boundary definition.

Table 5-1 Average thickness evaluation of three historical calvaria by external calliper measurement (MEA) and ray-tracing CT analysis (ANA).

Skull number/ Thickness (mm)	S1	S2	S3
MEA	3.74±0.82	4.54±0.95	6.52±1.05
ANA	3.66±0.95	4.46±1.01	6.58±1.00

5.2.5. Full skull characteristic maps

A total of 553,579 valid ray paths were obtained and remapped onto the skull surface, producing high-resolution target-specific maps of thickness, SDR, density, and sound speed (Figure 5-4). In the cranial vault, where the bone generally follows a layered and relatively smooth morphology, the method produced physically plausible maps. In contrast, the facial bones exhibited more complex non-layered geometry and were represented less accurately. Because tFUS is unlikely to be delivered through these regions, the full-skull maps are provided primarily to demonstrate the method.

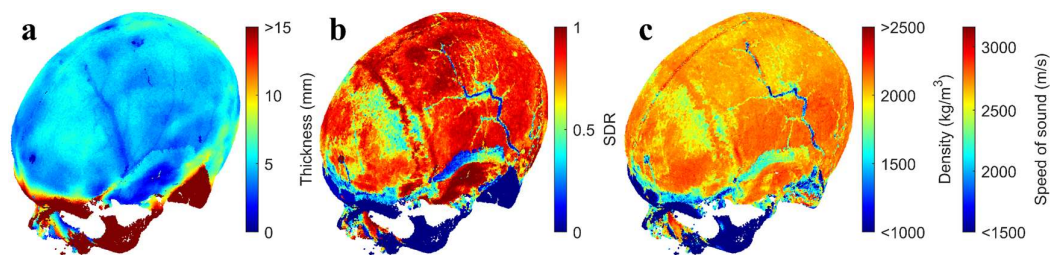


Figure 5-4 (a-c) Skull bulk thickness map, skull SDR map, and skull bulk density and sound speed map in respect of the Thalamic target.

As depicted in Figure 5-4.a, relatively thin regions are evident near the temporal window. These thickness values are target-specific: changing the intracranial target

changes the corresponding ray path and therefore the effective skull thickness along that path. The SDR map (Figure 5-4.b), highlights more porous regions around the temporo-parietal junction and vascular channels within the parietal bone, whereas the frontal-parietal suture appears comparatively uniform. These patterns are also reflected in the density and sound-speed map (see Figure 5-4.c).

The outer table, diploë, and inner table characteristics are shown separately in Figure 5-5. The two cortical layers appear relatively homogeneous, consistent with cranial anatomy, whereas the diploë is thicker and less dense in parts of the frontal, parietal, and temporal bones. These maps provide a macro-scale structural description relevant to transcranial propagation.

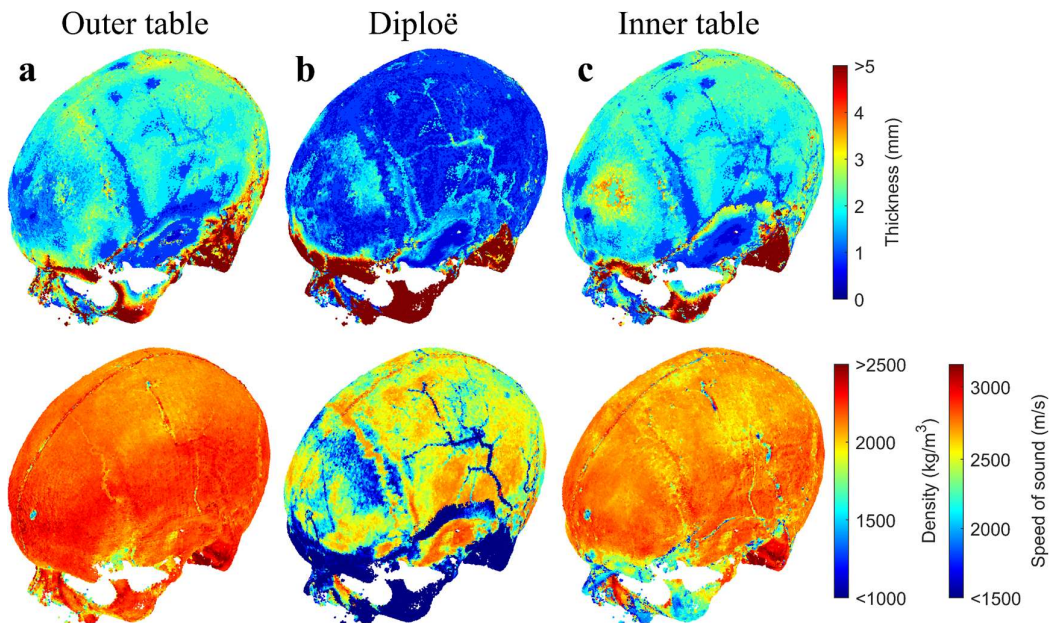


Figure 5-5 (a-c) The outer table, diploë, and inner table layer thickness, density and speed of sound characteristics.

5.2.6. Insertion angle map

Insertion angle is an important determinant of transcranial transmission because oblique incidence increases phase aberration and may enhance reflection and mode conversion. As shown experimentally in Section 4.5.1, near-normal incidence produced less distorted transmitted fields, whereas highly oblique incidence produced severe aberration and reduced transmission.

A target-specific insertion-angle map was therefore generated. For each ray, the local outer and inner skull-surface orientations were estimated by fitting a $5\text{ mm} \times 5\text{ mm}$ plane around the point of incidence and computing the corresponding surface normal. This neighbourhood was chosen to provide sufficient spatial averaging to reduce sensitivity to voxel-scale surface irregularities, while remaining small relative to the local skull curvature so that the surface could still be approximated as planar. Because the outer table is generally smoother than the inner table, the two local surface angles may differ substantially. The local insertion angle was therefore defined as the mean deviation between the incident ray and the outer- and inner-surface normals.

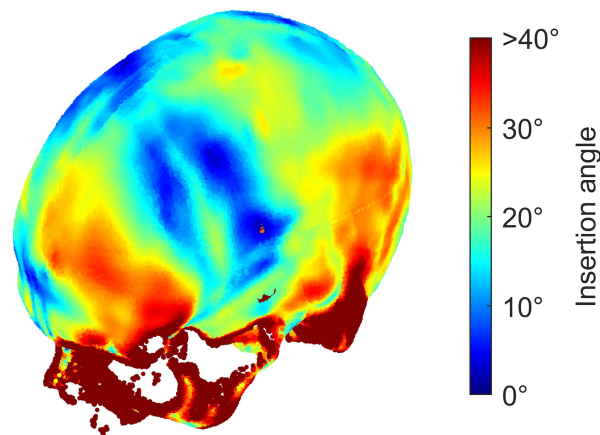


Figure 5-6 Ray insertion angle relative to the brain target.

As depicted in Figure 5-6, regions with relatively small insertion angle are located around the frontal-parietal-temporal junction, whereas sutures and more peripheral areas exhibit larger angles and are therefore less favourable for transmission. In practical use, transducer allocation may therefore be guided by joint consideration of insertion angle, skull thickness, SDR, and predicted transmission efficiency. Insertion angle may be used as an initial geometric constraint, particularly to avoid highly oblique incidence, while thickness and SDR can further distinguish suitable regions within this range.

5.3. Analytical model of transcranial transmission

The following sections develop a simplified analytical model for transcranial ultrasound transmission. The aim is to obtain rapid estimates of pressure insertion loss while retaining the principal wave interactions identified experimentally.

5.3.1. Monochromatic plane-wave approximation

For a small-aperture planar source, the simplest approximation is a continuous monochromatic plane wave propagating normal to the source surface, neglecting lateral spreading. Under these assumptions, the acoustic field in a homogeneous steady-state medium at rest may be written as

$$p = Ae^{i(kr-\omega t)}, \quad k = \frac{\omega}{c}, \quad (5.3.1)$$

where A is the wave amplitude. For a large-aperture focused transducer, however, the radiating surface cannot be represented adequately by a single plane-wave emitter. A more realistic approximation is to discretise the concave source into multiple small planar facets, such that the curvature over each individual facet can be treated as locally negligible, each with its own local propagation direction (Figure 5-7.b). The contributions from all facets are then combined to approximate the field generated by the complete concave aperture. The pressure field contributed by each facet may then be written as

$$p = Ae^{i(kxcos\theta + kysin\theta - \omega t)}. \quad (5.3.2)$$

Where θ is the oblique angulation. For simplicity, the elemental pressure amplitude was normalised to unity in the derivation.

5.3.2. Six-layer propagation framework

The architecture of the analytical model is described in this section. A concave PZ54 piezoceramic source ($c = 4300$ m/s and $\rho = 7800$ kg/m³, Ferroperm Piezoceramics, Denmark) directed toward the chosen brain target at a small incidence angle relative to

the skull is discretised into small planar facets (Figure 5-7.a and b). The skull is represented as a three-layer structure comprising outer table, diploë, and inner table. Water is included both upstream and downstream of the skull, and the transducer layer is retained explicitly, giving a six-layer total structure. (Figure 5-7.c).

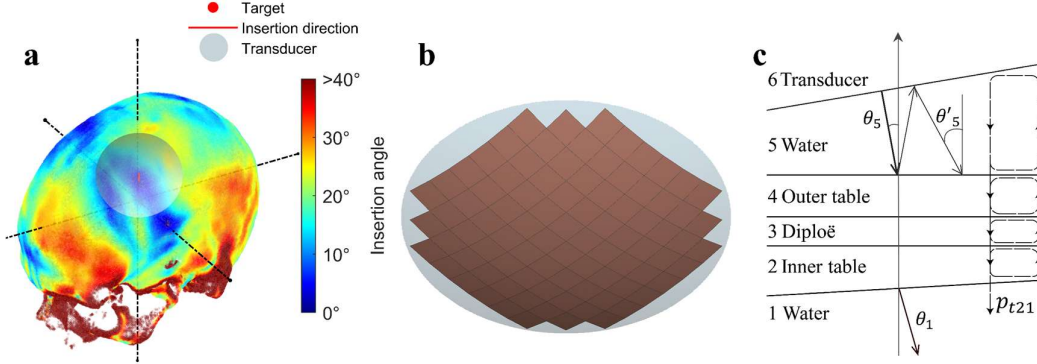


Figure 5-7 (a) A representative transducer arrangement relative to the brain target and the corresponding insertion direction. (b) Transducer face (blue shade) and its small flat-facets approximation. (c) Wave propagation scheme of the analytical model, arrows represent the direction of the propagation. p_{t21} represents the exit wave vector.

Using plane-wave assumptions, reflections and transmissions at each interface were modelled using boundary conditions determined by acoustic impedance, with each medium treated as locally semi-infinite. As shown in Figure 5-7.c, wave with a unit level of pressure is emitted from the transducer surface and progresses towards the outer table surface. The angulation between the two adjacent surfaces is determined by

$$\theta = \text{acos} \left(\frac{\vec{L}_i * \vec{L}_j}{|\vec{L}_i| * |\vec{L}_j|} \right), \quad (5.3.3)$$

where $\vec{L}_i$ and $\vec{L}_j$ represent the vector function of the boundaries i and j .

Upon the initial incidence on the boundary at an angle θ_5 , the horizontal propagation component was dropped to only consider the longitudinal mode, thus the wave propagation equation in the spatial domain can be simplified to the following form:

$$p_{i54} = e^{i\varphi_5}, \quad \varphi_i = k_i z_i \cos \theta_i, \quad k_i = \frac{\omega}{c_i}, \quad (5.3.4)$$

where p_{i54} is the original wave incident upon boundary 54, φ_i denotes to the distance z_i dependent phase advance, k_i is the wave number, ω is the angular frequency and c_i is the phase velocity.

The total inserted wave p_{t54} , after considering the portion that was directed back to the transducer surface and reflected towards the outer table again, consequently combines with the initial wave to form a superimposed wave, which can be written as:

$$p_{t54} = W_{54} e^{i\varphi_5} \sum_{n=0}^N (V_{54} V_{56} e^{2i\varphi'_5})^n, \quad (5.3.5)$$

where V_{ij} and W_{ij} are the reflection and transmission coefficient of the boundary ij .

Although a horizontal-infinite assumption was made where wave travels between two boundaries until it vanishes, in practice, the reflected energy decays significantly after two cycles. In an effort to reduce computing load, we set N equals to 2.

For wave emitted from fluid and impinging onto the elastic boundary, that is from water to the bone and transducer, the coefficients of longitudinal waves are (Brekhovskikh & Godin, 1990):

$$V_{ij} = \frac{Z_j \cos^2 2\theta_{js} + Z_s \sin^2 2\theta_{js} - Z_i}{Z_j \cos^2 2\theta_{js} + Z_s \sin^2 2\theta_{js} + Z_i}, \quad (5.3.6)$$

$$W_{ij} = \left(\frac{\rho_i}{\rho_j} \right) \frac{2Z_j \cos 2\theta_{js}}{Z_j \cos^2 2\theta_{js} + Z_s \sin^2 2\theta_{js} + Z_i}, \quad (5.3.7)$$

$$Z_i = \frac{\rho_i c_i}{\cos(\theta_i)}, \quad Z_j = \frac{\rho_j c_j}{\cos(\theta_j)}, \quad Z_s = \frac{\rho_j c_{js}}{\cos(\theta_{js})}, \quad i = 5, j = 4, 6, \quad (5.3.8)$$

where Z_i is the normal impedance of the medium, ρ_i is the density, θ_i is the normal angle of the wave in the layer, and θ_j and θ_{js} are the refracted angle of the longitudinal compression and shear (c_s) wave, and can be determined using Snell's law:

$$\frac{\sin \theta_i}{c_i} = \frac{\sin \theta_j}{c_j} = \frac{\sin \theta_{js}}{c_{js}}. \quad (5.3.9)$$

Here the longitudinal speed was used to derive the shear speed by multiplying it by a factor of 0.53 (P. J. White et al., 2006b).

The total penetrated wave undergoes another series of propagations in the outer table layer and leaves from boundary 43, waves which exit to the top water layer 5, reflected at boundary 56 and penetrated through 54 are not accounted as the magnitude of this portion of pressure is considered negligible. Therefore, the calculation of transmission through boundary 43 is carried out using the same equation. While in the calculation of the total transmission through layer 32, it is imperative to consider the portion of wave that is reflected at boundary 32, subsequently back propagated through boundary 34, reflected off at boundary 45, transmitted through boundary 43, and finally re-incident upon boundary 32. To determine the total transmission through boundary 32, the equations are expressed as the sum of the original wave p_{t32_orig} and the reflected wave p'_{t32} from boundary 45.

$$p_{t4_orig} = p_{t54} W_{43} e^{i\varphi_4 a_4} \sum_{n=0}^N (V_{43} V_{45} e^{2i\varphi_4 a_4^2})^n, \quad (5.3.10)$$

$$p_{t32_orig} = p_{t43_orig} W_{32} e^{i\varphi_3 a_3} \sum_{n=0}^N (V_{32} V_{34} e^{2i\varphi_3 a_3^2})^n, \quad (5.3.11)$$

$$p_{r34} = p_{t43_orig} V_{32} W_{34} e^{2i\varphi_3 a_3^2} \sum_{n=0}^N (V_{32} V_{34} e^{2i\varphi_3 a_3^2})^n, \quad (5.3.12)$$

$$p_{r45} = p_{r34} V_{45} W_{43} e^{2i\varphi_4 a_4^2} \sum_{n=0}^N (V_{43} e^{2i\varphi_4 a_4^2})^n, \quad (5.3.13)$$

$$p'_{t43} = \frac{p_{r45} p_{t43_orig}}{p_{t54}}, \quad (5.3.14)$$

$$p'_{t32} = \frac{p'_{t43} p_{t32_orig}}{p_{t43}}, \quad (5.3.15)$$

$$p_{t32} = p_{t32_orig} + p'_{t32}, \quad (5.3.16)$$

Where a_4, a_3, a_2 represent the attenuated coefficient in outer table, diploe and inner table, respectively.

In the same manner, the total transmission at the boundary 21 can be written as the sum of the original wave p_{t21_orig} and the reflected waves p'_{t21} and p''_{t21} from boundary 34 and 45, respectively.

$$p_{t21} = p_{t21_orig} + p'_{t21} + p''_{t21}. \quad (5.3.17)$$

The full coefficient expressions for the skull-water and skull-skull interfaces are retained in the APPENDIX E. The main text describes the physical sequence of reflections and transmissions, while the complete algebraic derivation of p_{t21} is not shown due to the substantial amount of redundant content.

5.3.3. Full-skull transmission efficiency estimation

Before incorporating the transducer explicitly, full-skull transmission efficiency was first estimated using skull properties alone. For each ray, transmission efficiency was calculated at 250 kHz, 500 kHz, 750 kHz, and 1000 kHz. These maps provide a first-pass indication of where low insertion angle and favourable transmission coincide, and thus where transducer placement may be advantageous.

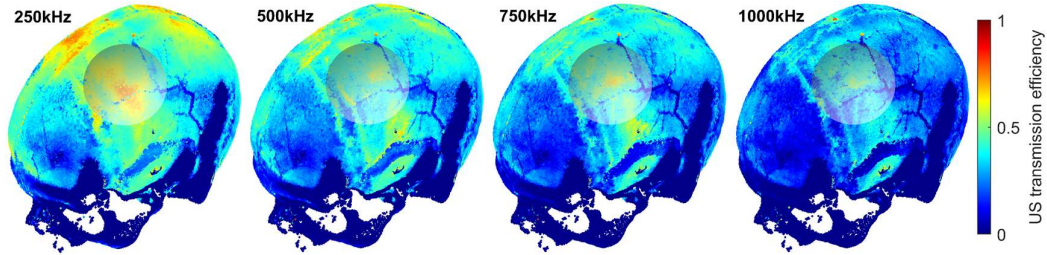


Figure 5-8 Ultrasound pressure transmission efficiency across the skull surface at four typical frequencies. Transducer layer was not taken into account.

As shown in Figure 5-8, regions of higher predicted transmission generally correspond to skull regions with favourable thickness, SDR, and insertion angle. However, different skull regions also show different optimal transmission frequencies. The full-skull map should therefore be interpreted not as a single universal placement guide, but as a frequency-dependent atlas of target-specific transcranial accessibility.

5.4. Model validation

The transmission-efficiency maps described above consider skull characteristics alone and are therefore conceptually similar to previously proposed skull-only analytical approaches. However, they do not represent the full system insertion loss, because they exclude the physical transducer and the water gap in which spacing-dependent interference may arise. As demonstrated experimentally in Section 4.5.2, This gap can strongly influence intracranial pressure, particularly at lower frequencies. To capture this, the full analytical solution explicitly includes transducer geometry so that wave interactions throughout the entire transmission path are preserved.

The analytical model was validated against both k-Wave simulation and hydrophone measurement. Validation was performed by first verifying the numerical source implementation in free-field, and then cross-validation against ex-vivo skull measurements.

5.4.1. k-Wave solver

A particular advantage of k-Wave is that off-grid source interpolation can reduce staircase artefacts in bowl-shaped source masks. However, the default off-grid source formulation treats the source as acoustically transparent, allowing waves to pass through the source region unrealistically. To preserve physically meaningful reflections in the present study, a piezoceramic-like layer was therefore placed at the source location using approximate material properties of $v = 4300$ m/s and $\rho = 7800$ kg/m³.

To balance reduction of staircase artefacts against excessive source thickening, the band-limited interpolant (BLI) tolerance was set to 0.9, leaving a source geometry three voxels thick (Figure 5-9). This provided a practical compromise between source smoothness and preservation of a well-defined transducer surface. Because this arrangement substantially reduced emitted pressure (approximately 94% to 95%) as the wave entered water owing to the impedance mismatch between the piezoelectric

layer and the surrounding medium, a compensatory scaling factor was introduced to restore the intended free-field source strength.

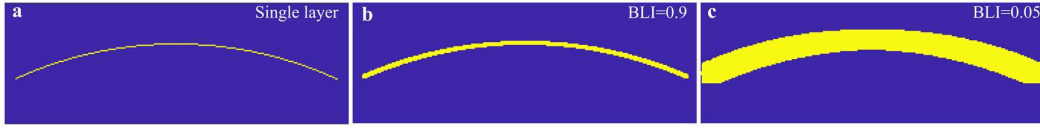


Figure 5-9 (a) The original source surface layer ($D = 60$ mm, $RoC = 75$ mm). (b and c) The source signal mask with BLI number set to 0.9 and 0.05 (default), respectively.

A grid resolution of 30 points per wavelength in water was used for the free-field focal-gain benchmark. The resulting axial focal-gain curves are shown in Figure 5-10 and compared with the O’Neil analytical solution (Section 3.1.4). The k-Wave model reproduced close agreement at the far-field focus, although some near-field deviations remained when the piezo layer was included. These deviations were accepted because the focal-gain agreement indicated that no major systematic setup error was present, and the same source treatment was then retained for the subsequent validation studies.

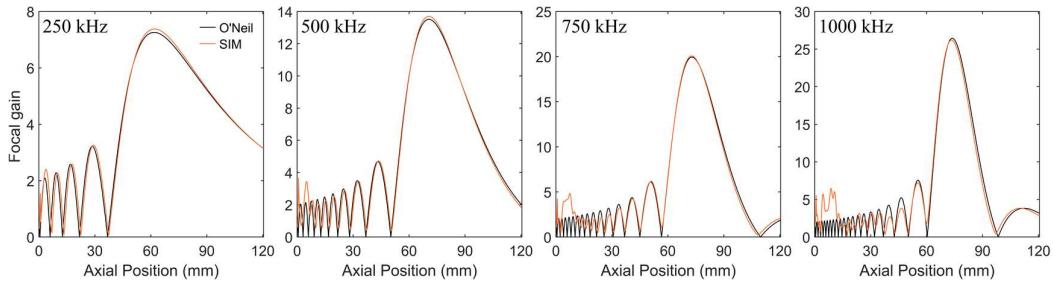


Figure 5-10 Axial focal gain when simulating with a piezo layer. The simulated results (SIM) were compared with the O’Neil analytical solution at 250 kHz, 500 kHz, 750 kHz and 1000 kHz.

5.4.2. Benchmark in simplified layered geometry

Before applying the model to skull data, a simplified benchmark was performed against k-Wave simulation in order to test whether the analytical model could reproduce wave-superposition behaviour in a layered medium. The free-field simulation domain was modified by adding two 5 mm solid plates ($v = 2700$ m/s and $\rho = 1200$ kg/m³) separated by a 5 mm water gap, while retaining the focused source above the slab. (see Figure 5-11.a), and the steady-state peak pressure in the downstream of the slab was recorded. The simulation was run in a fluid solver with attenuation set to zero and repeated from

100 kHz to 1000 kHz in 10 kHz increments, giving 91 frequency steps in total. The downstream peak pressure was normalised to the free-field value and compared with the analytical prediction under the same geometry.

As shown in Figure 5-11.b, pronounced wave interactions developed within and between the layers, confirming the importance of modelling each layer explicitly in order to recover transmitted peak pressure accurately.

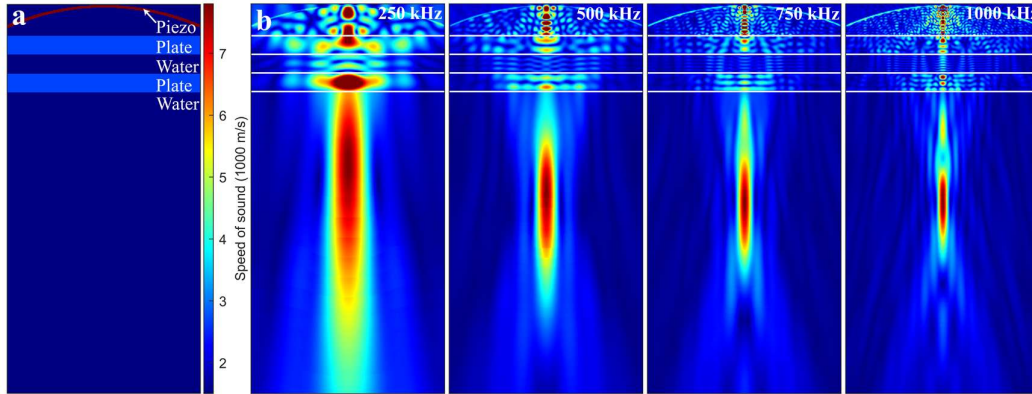


Figure 5-11 (a) k-Wave simulation domain with the mediums sound speed setup. (b) The simulated normalised field at four typical frequencies using the three-layer slab.

Figure 5-12 shows that the analytical and simulated pressure-transmission curves shared the same overall oscillatory pattern, although the analytical result produced somewhat stronger constructive and destructive extrema, suggesting slight overestimation of coherent wave summation. Nevertheless, the agreement in trend indicates that the layered analytical model is capable of reproducing the principal superposition effects in homogeneous layered media.

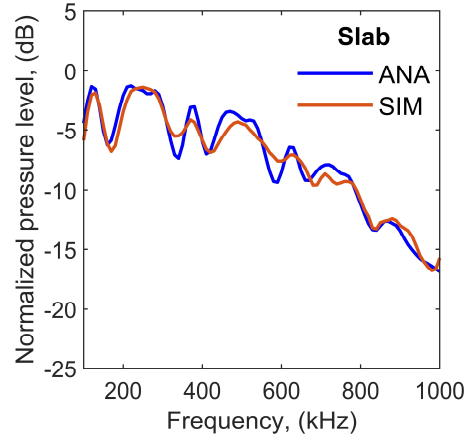


Figure 5-12 Calculated (ANA) and simulated (SIM) normalised peak pressure level across 100 kHz to 1000 kHz frequency.

5.4.3. Experimental validation setup

Hydrophone validation was performed using 220 kHz and 680 kHz transducers swept from 100-290 kHz and 300-1000 kHz, respectively, in 10 kHz increments. Bursts of 100 and 150 cycles were delivered using a function generator and an amplifier (1020L amplifier, E&I, US), and focal peak pressure was measured in free-field using a 1 mm needle hydrophone (Precision Acoustics, UK). Input power was increased at off-resonant frequencies to maintain measurable signal amplitude across the sweep, with at least -25 dB coverage within each band. Signals were band-pass filtered and windowed at the input frequency using a 10 Hz gate.

The mean free-field peak signal amplitudes across the two sweep ranges were 604.5 mV (0.81 MPa) and 1460.5 mV (1.57 MPa), with minimum amplitudes of 28.4 mV and 75.4 mV, respectively (see Figure 5-13).

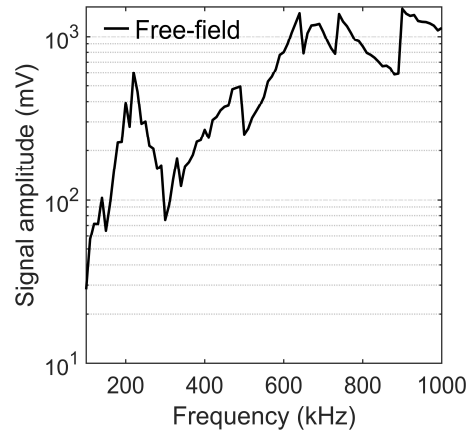


Figure 5-13 Signal amplitude measured using 1 mm hydrophone in free-field, frequency sweep from 100 to 290 kHz using 220 kHz transducer, and 300 kHz to 1000 kHz using 680 kHz transducer.

For the 220 kHz transducer, the usable bandwidth below 150 kHz was limited by its narrow resonant response; even doubling the input voltage did not provide sufficient signal gain for reliable measurements, and the lower bound was therefore set at 100 kHz.

For transcranial measurements, each hydrated and vacuum-degassed skull RoI was positioned approximately 10 mm from the transducer aperture. The same sonication sequence was repeated ten times. After each frequency sweep, the skull was displaced incrementally away from the transducer by 0.2-0.4 mm in order to sample spacing-dependent interference.

5.4.4. Cross-validation in ex-vivo skulls

Twenty ex-vivo historical skulls (19 calvaria and 1 half skull, labelled as S1-S20) were used for validation. All skulls were scanned in the hydrated condition using CT scanner (Revolution EVO, GE, US) with a voxel size of $0.44 \times 0.44 \times 0.62 \text{ mm}^3$ and a BonePlus convolution kernel at 120 kVp for enhanced bone delineation. The corresponding bulk thickness, density, and SDR maps are provided in APPENDIX D.

For each skull, a target approximating the thalamus was defined, and one skull region predicted to provide relatively favourable insertion conditions was selected (Figure 5-14). These regions were segmented and analysed across frequencies from 100 kHz to 1000 kHz in 10 kHz steps and across transducer-skull spacings from 10 mm to 20 mm

in 0.1 mm increments, producing 100 analytical repeats per RoI. On an Intel i7-12700K CPU, each full analytical sweep for one RoI completed within seconds.

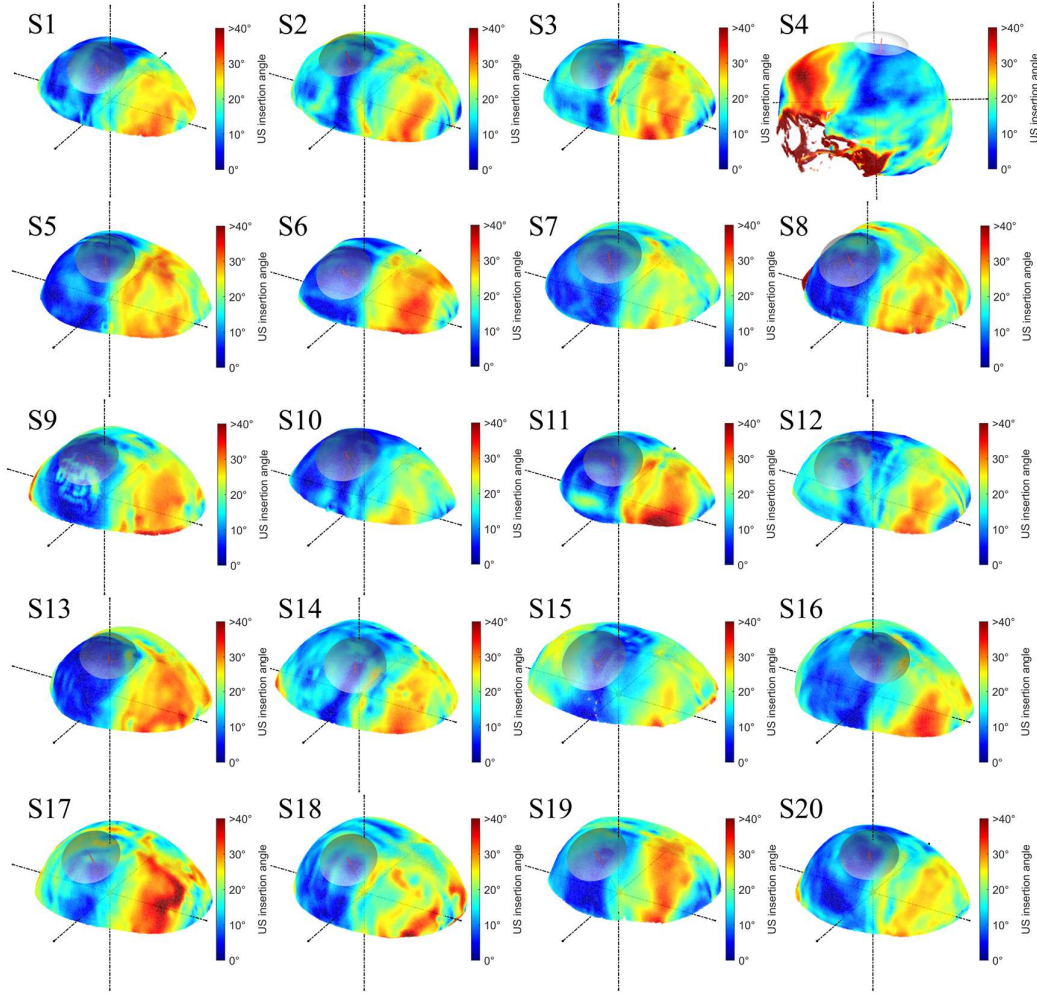


Figure 5-14 Ultrasound insertion angle map with transducer placement of S1 to S20. This angle is averaged over the inner and outer skull surface angle, and prior to taking ultrasound emission angle into account.

Equivalent k-Wave simulations were then constructed. A 3D grid of $144 \times 144 \times 196$ voxels with 0.44 mm spacing was used, and the selected skull region was inserted together with a concave piezoceramic layer at the source location. Simulations were run in a fluid solver using 18 points per wavelength, BLI tolerance 0.9, and a Courant–Friedrichs–Lewy number of 0.15 per MHz. Peak focal pressure was recorded both in free-field and with the skull present. Five repeats were performed at each frequency, with the skull shifted by one voxel after each run to sample spacing-dependent

interference. In total, this produced 455 simulations and required approximately 46 h on an NVIDIA RTX 3090 Ti GPU.

Experimentally, each hydrated and vacuum-degassed skull RoI was placed in the corresponding geometry and measured using the same frequency-sweep protocol, with ten repeated sonications per sweep and incremental transducer-skull spacing shifts to sample interference.

Three insertion-loss curves were then obtained for each RoI: analytical (ANA), simulation (SIM), and measurement (MEA). Statistical analysis was performed in SPSS v26.0 and MATLAB. Frequency-dependent pressure-loss deviations and percentage errors (ε) were calculated for each pair of curves

$$\varepsilon(x, MEA) = \frac{p_x - p_{MEA}}{p_{MEA}} * 100\%, \quad x \in [ANA, SIM],$$

$$\varepsilon(ANA, SIM) = \frac{p_{ANA} - p_{SIM}}{p_{SIM}} * 100\%,$$

where p is the normalised intracranial peak pressure. The pressure loss level uncertainty (u) at each frequency was estimated as the average relative deviation of the maximum and minimum values from the mean, expressed as a percentage:

$$u_i = \frac{|p_{i,max} - \bar{p}_i| + |p_{i,min} - \bar{p}_i|}{2\bar{p}_i} * 100\%.$$

The result was reported as the median and interquartile range (IQR). Monotonic agreement was assessed with Spearman correlation (r_s):

$$r_s = 1 - \frac{6\sum d_i^2}{n(n^2 - 1)},$$

where n is the number of paired frequencies ($n=91$), and d is the difference between the ranks of the paired data with significance threshold of $p < 0.05$. Oscillatory similarity was evaluated by computing the cosine similarity (CS) between the slope vectors of each two paired curves:

$$CS = \frac{\sum_{i=1}^n A_i \cdot B_i}{\sqrt{\sum_{i=1}^n A_i^2} \cdot \sqrt{\sum_{i=1}^n B_i^2}},$$

where A, B are vectors of the two test results, n is the number of frequencies ($n=91$), and CS represents the cosine similarity. Depending on the outcome of the normality test, results are reported as mean $\pm$ standard deviation (std), or as median values with IQR.

5.5. Validation results

5.5.1. Frequency-dependent insertion loss prediction

The 20 validation RoIs varied substantially in physical properties, with mean thickness 6.4 ± 1.8 mm, SDR of 0.53 ± 0.19 and CT bulk density of 958 ± 212 HU. The average insertion angle across the effective incident area was $9.0 \pm 1.5^\circ$. Figure 5-15 compares analytical, simulated, and measured pressure insertion-loss curves from 100 kHz to 1000 kHz. Although empirically calibrated attenuation and sound-speed relations were used, some trend outliers remained, most notably S18.

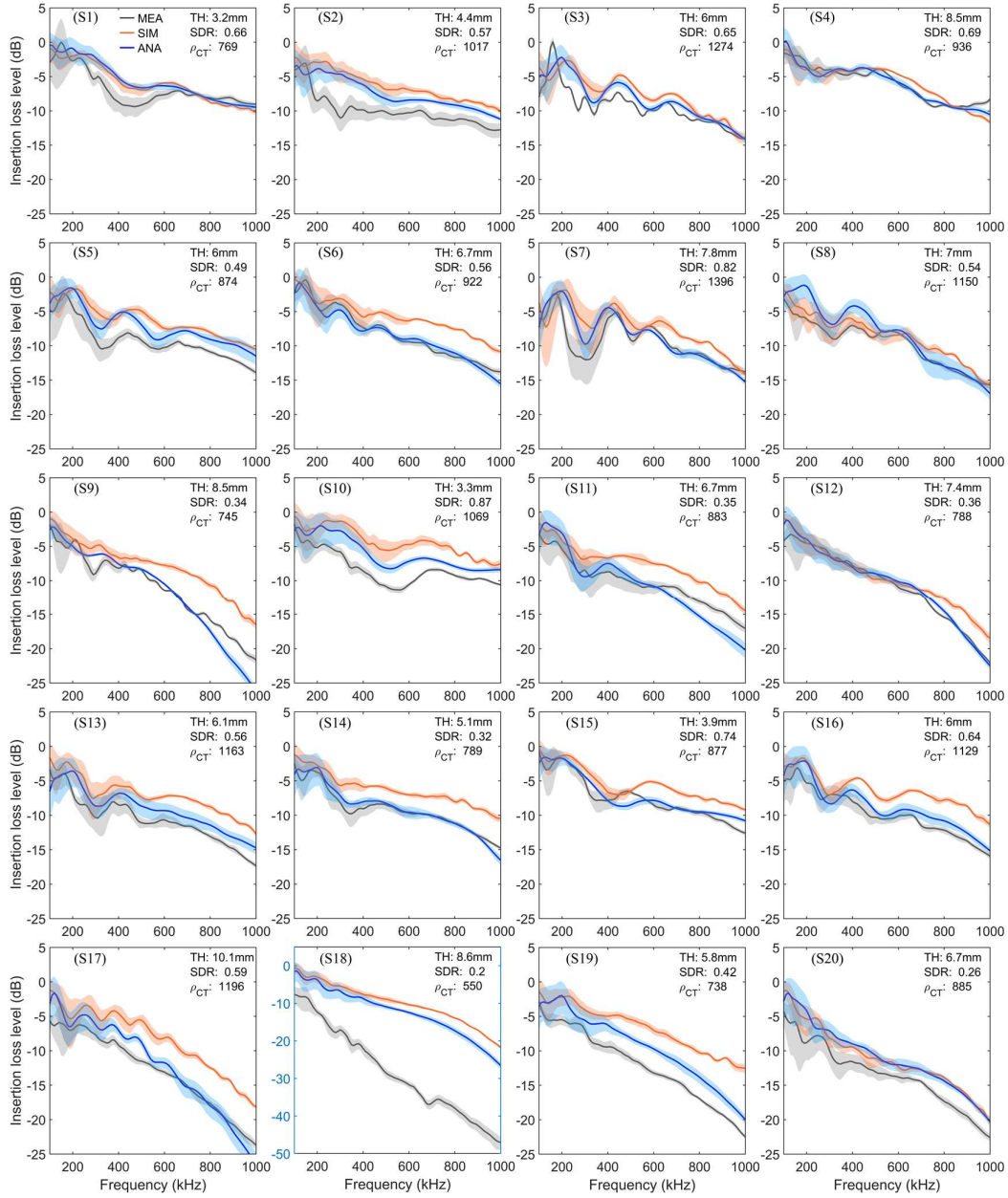


Figure 5-15 Measured, simulated and analysed pressure insertion loss levels of 20 skulls' RoI (reported in decibels). The corresponding pressure insertion loss levels in percentage are provided in APPENDIX F.

Insertion loss exhibited pronounced oscillation at lower frequencies, particularly below approximately 700 kHz, in more than half of the RoIs. This behaviour is consistent with constructive and destructive wave interference within the skull and in the transducer-skull gap. Oscillation amplitude depended on multiple morphological factors, including thickness variability, RoC, and possibly high bulk density or high SDR. At the group

level, as shown in Figure 5-16.a, the generalized linear mixed-model fits for insertion loss (IL, dB) are:

$$IL_{MEA} = -13.9F - 3.5, \quad R^2 = 0.69;$$

$$IL_{ANA} = -14.8F - 1.0, \quad R^2 = 0.73;$$

$$IL_{SIM} = -10.9F - 1.3, \quad R^2 = 0.75;$$

Relative to measurement, the analytical model predicted pressure insertion-loss magnitude with a median deviation of +1.1 dB (IQR: +0.2 dB to +2.2 dB, Figure 5-16.b) and a median percentage error of +30.1% (IQR: +9.5% to +35.6%, Figure 5-16.c). This supports the use of a layered skull representation with dynamic density-dependent attenuation, although the systematic positive bias indicates that the model tended to overestimate insertion loss. For the present dataset, the upper-quartile deviation of 2.2 dB (approximately 29% in pressure) provides a practical modelling allowance when interpreting predicted intracranial pressure, but should not be regarded as a universal safety factor.

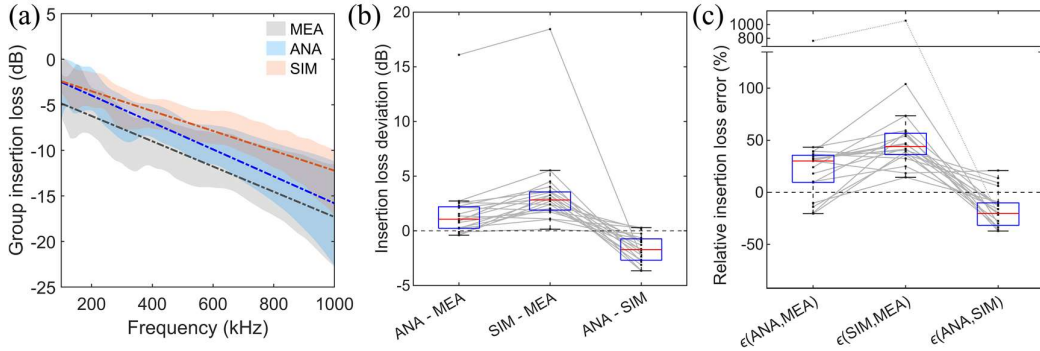


Figure 5-16 (a) Group insertion loss averaged over 20 skulls' RoI. (b) Inter-group pressure insertion loss deviation between each two results. ANA - MEA: median: +1.1 dB, max: +16.1 dB, min: -0.4 dB; SIM - MEA: median: +2.8 dB, max: +18.4 dB, min: +0.2 dB; ANA - SIM: median: -1.7 dB, max: +0.3 dB, min: -3.7 dB. (c) Inter-group pressure insertion loss error in percentage: $\epsilon(\text{ANA}, \text{MEA})$: median: +30.1%, max: +762.3%, min: -20.5%; $\epsilon(\text{SIM}, \text{MEA})$: median: +44.0%, max: +1057.0%, min: +14.4%; $\epsilon(\text{ANA}, \text{SIM})$: median: -20.3%, max: +20.9%, min: -37.2%.

The strongest outlier was S18 (TH=8.6mm, SDR=0.2, ρ_{CT} =550), a highly heterogeneous skull region in which the analytical error reached +762.3% relative to measurement (Figure 5-16.c). By contrast, the remaining 19 RoIs showed percentage

errors between -20.5% and +43.3%. This suggests that strongly heterogeneous local structure may violate the assumptions of the present three-layer approximation.

The simulation underestimated attenuation relative to both measurement and the analytical model, with median deviation of +2.8 dB (IQR: +1.9 dB to +3.6 dB) and median percentage error of +44.0% (IQR: +36.2% to +56.7%), compared to measurement, and +1.7 dB (IQR: +0.7 dB to +2.7 dB) and +20.3% (IQR: +10.1% to +31.7%) to analytical model.

5.5.2. Oscillatory behaviour and spacing uncertainty

Figure 5-17.a illustrates the sensitivity of insertion loss to transducer-skull spacing. At low frequency, for example 150 kHz, shifting the skull by only a few millimetres altered measured insertion loss from -5.3 dB to +2.9 dB. This sensitivity decreased with increasing frequency and stabilised to approximately ± 0.6 dB roughly beyond 650 kHz, where absorption effects dominate interference. The median pressure loss uncertainty in percentage across 20 RoIs is 14.1% (IQR: 10.0% to 18.4%, Figure 5-17.b). The simulation reproduced the overall trend but sampled fewer spacing and therefore underestimates the peak uncertainty, with a median uncertainty in percentage of 10.4% (IQR: 8.9% to 12.3%). In contrast, the analytical model, evaluated at 0.1 mm increments, predicted average uncertainties of -2.0 dB to +1.4 dB, marginally higher than the simulation and measurement, and with uncertainty in percentage of 18.1% (IQR: 17.2% to 21.3%). These findings demonstrate that the physical presence of the transducer and the water-gap spacing materially affect transmitted pressure, particularly at lower frequencies.

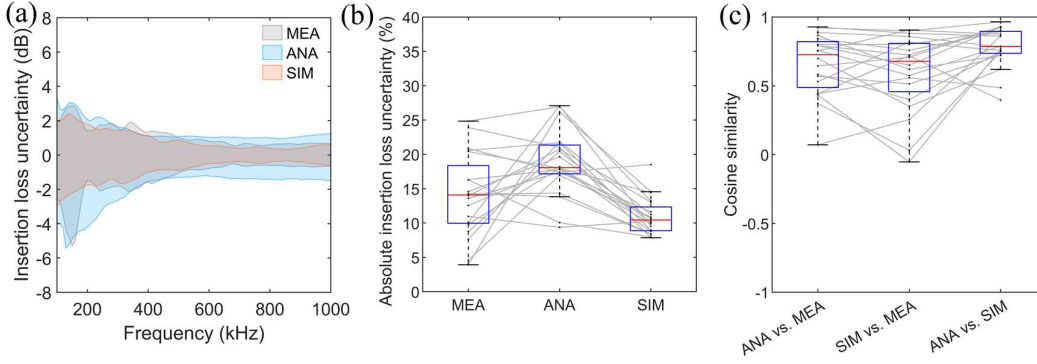


Figure 5-17 (a) Average insertion loss uncertainties caused by varying transducer-skull spacing over 20 skulls RoI. (b) Absolute insertion loss uncertainty averaged over frequency: MEA: median: 14.1%, max: 24.8%, min: 3.9%; ANA: median: 18.1%, max: 27.1%, min: 9.4%; SIM: median: 10.4%, max: 18.5%, min: 7.9%. (c) Inter-group comparison of cosine similarity of each two result curves. ANA vs. MEA: median: 0.73, max: 0.93, min: 0.07; SIM vs. MEA: median: 0.68, max: 0.91, min: -0.05; ANA vs. SIM: median: 0.79, max: 0.97, min: 0.40.

Analytical predictions reproduced the oscillatory pattern that in general agreement with the measurements (median $r_s = 0.92$, IQR: 0.86 to 0.98, $p < 0.001$; median CS = 0.73, IQR: 0.49 to 0.82; Figure 5-17.c). Simulation exhibited similar but slightly weaker agreement (median $r_s = 0.91$, IQR: 0.86 to 0.98, $p < 0.001$; median CS = 0.68, IQR: 0.46 to 0.81). Trending mismatches obviously occurred at S15 and S17 in both predicting methods, suggesting that speed of sound and layers were not accurately represented. Stronger correlation is evident between simulation and analytical results (median $r_s = 0.97$, IQR: 0.96 to 0.99, $p < 0.001$; median CS = 0.79, IQR: 0.74 to 0.90), suggesting that the wave phase modelling framework captured the principal interference behaviour almost as effectively as the full-wave numerical solution.

5.6. Discussion

This chapter presents a CT-informed, six-layer analytical model for predicting tFUS pressure insertion loss. Cross-validation against k-Wave simulations and hydrophone measurements in 20 ex-vivo skull samples confirms that the framework reproduces key interference phenomena across sub-MHz frequencies while remaining computationally tractable. High correlation among the three techniques underscores the value of layered,

acoustic impedance-based approach for rapid insertion loss estimation during tFUS planning. Notwithstanding, this study was conducted using ex-vivo skulls, which may not fully capture the diversity of in-vivo cranial conditions. Although all ex-vivo skulls were rehydrated prior to testing, differences in marrow composition and microstructures may alter acoustic transmission relative to living tissue. In addition, the absence of surrounding tissues, including scalp, dura mater, and brain tissue limits direct translational applicability. Future work should incorporate more physiologically realistic bone models, such as freshly excised or realistically embalmed skulls to better assess clinical applicability.

One of the most notable findings was the prevalence of constructive and destructive interference patterns, producing oscillatory insertion loss curves in both measurements and predictions. Such interference pattern arises from multiple reflections at the water-transducer interface, the skull-water interface, and the internal interfaces between skull layers (Aarnio et al., 2005). Beyond approximately 700 kHz, the oscillation amplitude decreases as absorption governs. Because sub-MHz frequencies are favoured for neuromodulation (deeper penetration with minimal heating), recognising these interference patterns is essential for dose control. In this study, the patterns were observed at short transducer-skull spacings, representing conditions for targeting deeper brain regions such as the thalamus. Superficial cortical targets are also of interest, and prior work showed that qualitative interference patterns remained consistent across both short (10 mm to 20 mm) and long (40 mm to 50 mm) spacings, suggesting that oscillatory behaviour would also occur when targeting more superficial structures. Although the analytical model was evaluated the spacing effect at fine increments of 0.1 mm, practical considerations limited the simulations to 0.44 mm, as each frequency spectrum sweep required approximately 10 hours to complete.

In summary, the six-layer analytical model proposed here integrates skull thickness and SDR, ultrasound insertion angle, and transducer geometry to yield insertion loss predictions that generally align closely with both simulation and experimental measurements. The three sets of results show moderate to high oscillatory similarity and high trending correlations. Additionally, the required computing time for analytical model is in the matter of hours. This balance of accuracy and computational efficiency

positions the model as a valuable tool for tFUS neuromodulation procedure planning—one that can inform transducer placement, predict optimal frequency usage, and reduce the variability in delivered ultrasound energy.

5.6.1. Comparison with other bone modelling strategies

Several analytical studies used single-layer representations with spatially averaged properties, which recovered fluctuation trends in energy transmission (Attali et al., 2023)(P. J. White et al., 2006a). Fry (F. J. Fry & Barger, 1978) proposed a three-layer bone model, quantifying phase speed and phase angle in different layers, and reporting a diploe-specific loss coefficient of $11.5 \text{ dB}/(\text{MHz}^4 \cdot \text{cm})$ based on the squared difference of bulk modulus between the blood-bone matrix and blood-fat matrix. Hayner (Hayner & Hynynen, 2001) further expanded the solution to encompass an additional scalp layer and incorporated oblique ultrasound incidence. While these models demonstrated that intracranial pressure maxima occur at skull resonance frequencies, none accounted for the physical presence of the transducer or for wave super-position in the intervening water path.

The present work extends this lineage by introducing the transducer modelling, discretising the aperture into differential area and allowing wave to interact between transducer-skull spacing. This yields a more comprehensive tFUS transmission domain without incurring the prohibitive run-times of full-wave solvers.

Nevertheless, the three-layer skull representation used here remains a simplification of cranial bone heterogeneity. To partially address spatial variability, we employed a voxel-wise adaptive three-layer model, allowing adjacent rays to traverse distinct local layer thicknesses and property assignments. More adaptive segmentation strategies, such as dynamic multi-layer skull modelling, could in principle improve fidelity in regions with complex anatomy (e.g., the squamosal suture where overlapping bone violates the three-layer assumption, or in diploë containing interdigitating vasculature), however, analytical complexity would increase substantially, and accurate delineation is further constrained by the limited resolution of current clinical CT. This trade-off may explain why most existing skull analytical models have been restricted to three layers, which remain sufficient in many cases. Severe local variations in porosity and

microarchitecture, as observed in S18, can nevertheless lead to large discrepancies in insertion loss predictions, indicating that strongly anisotropic and heterogeneous cranial bone cannot be faithfully represented by layered decomposition alone. Due to the limited number of specimens, we could not quantify a structural heterogeneity threshold beyond which prediction errors become unacceptable. But it shall be reasonable to assume that thick or highly heterogeneous skulls pose greater challenges.

Two factors may help explain why the simplified three-layer representation remained largely effective in this study. First, the sub-MHz frequency range employed here yield wavelengths several times larger than typical trabecular bone spacing (approximately 0.83 mm in highly porous regions (Chaffai et al., 2002)), which reduces but does not eliminate microstructure-induced scattering. Second, the near-normal incidence reduced the likelihood of refraction-driven shear mode conversion. In instances where the three-layer approximation is inadequate, full-wave simulations may provide an alternative; although their accuracy is also constrained by the wide variability in reported correlations between HU and acoustical parameters. Such uncertainty may partly arise from differences in CT acquisition parameters (Aubry et al., 2003). In our work, the simulation model tended to underestimate attenuation compared to both measurements and the analytical model. This discrepancy is unlikely to arise from insufficient attenuation coefficient settings alone, a potential cause may instead stem from modelling the domain as purely fluid mediums rather than viscoelastic, although the significance has not yet been verified due to the significant increase in time investment. Despite these challenges, both the analytical and simulation models demonstrated pressure insertion loss predictions that generally trended with hydrophone measurements.

Table 5-2 compares 25% to 75% interquartile range of peak transmitted pressure ratio obtained by MEA, ANA and SIM with published values at four typical (or similar) frequencies. Discrepancies across studies are attributable to variability in skull morphology, transducer geometry and driving waveform. It is worth noting that, because the proposed analytical model generally underestimated pressure insertion loss, compensating for this bias would require increasing the applied pressure to achieve a desired in-situ value. However, given the variability in prediction errors (-20.5% to

+43.3%, with one outlier at +762.3%) and uncertainty from transducer-skull spacing (9.4% to 27.1%), such adjustments risk overshooting guideline safety limits for mechanical index or acoustic intensity in individual cases. Therefore, the analytical model is better interpreted as a relatively conservative predictor, suitable for supporting treatment planning while recognizing that deviations in either direction can still occur. Safe application should also involve verification against maximum allowable safety limits at the corresponding frequency, with adjustments made downward as needed.

Table 5-2 Peak pressure transmission ratio comparison with other studies. Literatures unspecified to cycles of burst, skull thickness and density, or transducer characteristics.

	MEA	ANA	SIM	MEA	ANA	SIM
	Peak pressure transmission ratio					
Frequency	Present study, Interquartile range			Literature		
250 kHz	[34% 57%]	[47% 65%]	[57% 76%]	32%, 270 kHz (Gimeno et al., 2019); 46%, 270 kHz (Krokhmal et al., 2025)	72%, 200 kHz (Attali et al., 2023)	49%, 250 kHz (W. Lee et al., 2015b); 47%, 250kHz (Hosseini et al., 2023); 43%, 270 kHz (W. Lee et al., 2016)
500 kHz	[28% 38%]	[35% 43%]	[41% 52%]	70%, 500 kHz (Legon et al., 2018c); 50%, 500 kHz (Legon et al., 2014); 31%, 500 kHz (Krokhmal et al., 2025)	62%, 500 kHz (Attali et al., 2023)	65%, 500 kHz (Yaakub et al., 2023); 29%, 500 kHz (Strohman et al., 2024); 39%, 500 kHz (Hosseini et al., 2023)
750 kHz	[17% 31%]	[21% 35%]	[33% 42%]	17%, 750 kHz (M. Chen et al., 2023); 21%,	35%, 700 kHz (P. J. White et al., 2006a); 23%, 750 kHz (Hayner &	31%, 750 kHz (Hosseini et al., 2023)

				750 kHz (Krokhmal et al., 2025)	Hynynen, 2001); 57%, 800 kHz (Attali et al., 2023)	
1000 kHz	[8% 23%]	[9% 27%]	[16% 30%]	15%, 1000kHz (Pinton et al., 2012b); 14%, 1000 kHz (Krokhmal et al., 2025)	53%, 1000 kHz (Attali et al., 2023); 15%, 1000 kHz (Guo et al., 2022b)	15%, 1000kHz (Pinton et al., 2012b)

The present validation was limited to an upper frequency of 1000 kHz by the bandwidth constraint of the transducers employed. While this range covers the majority of clinical tFUS studies, higher frequencies above 1000 kHz are also used in neuromodulation studies (Di Biase et al., 2019). Extending validation into this range would enhance translational relevance. Future studies could employ broader-bandwidth transducers to enable reliable testing at these higher frequencies.

The present model emphasizes longitudinal transmission but allows for shear-wave excitation through the use of an elastic-wave formulation. Because the transducer surface was positioned nearly normal to the skull surface, shear contributions were expected to be minimal in the central aperture. At more oblique incidence near the periphery, however, partial conversion into shear mode likely occurs. Although this effect is implicitly included, the model does not explicitly quantify the proportion of energy transmitted through as shear waves, which may lead to higher insertion loss prediction uncertainty at larger incidence angles.

5.6.2. Practical implications for transducer allocation

Existing transducer placement methods either minimise surface reflection from the skull surface (Park et al., 2019), simulating intracranial ultrasound pressure attenuation (Gao et al., 2023), or by visually inspecting the back-propagated US phase map from the brain target (Butler et al., 2022). By integrating the ultrasound insertion angle, skull thickness, SDR and transmission efficiency maps, our pipeline produces a full-scale

skull evaluation. A complete atlas generation consumed approximately 25 hours for a full skull and 6 hours for calvaria by using i7-12700K CPU. In comparison, a full-scale k-Wave simulation for a skull at one frequency requires nearly 500 hours (Gao et al., 2023). This makes the method suitable not only for retrospective analysis, but also for practical pre-planning of transducer allocation and frequency selection.

.

6. Full-wave modelling and in-vivo evaluation

The analytical framework developed in the previous chapter provides a rapid method for estimating transcranial insertion loss and screening transducer placement. However, insertion loss alone is not sufficient to characterise the intracranial acoustic field relevant to neuromodulation, and full-wave modelling remains necessary to resolve volumetric coverage within and around the intended target. This chapter therefore first considers intracranial field prediction, with the performance of five modelling approaches evaluated against measurements acquired in ex-vivo skull specimens. The chapter then turns to a preliminary system-evaluation study, asking whether a physically characterised tFUS setup can be integrated with hippocampal electrophysiology while preserving interpretable recordings.

6.1. Introduction

Full-wave modelling is required not only to estimate how much acoustic energy passes through the skull, but also to determine how that energy is distributed within and around the intended target. In the present study, the acoustic source was defined from the measurement-informed reconstruction described in Chapter 3, the remaining major uncertainty therefore lies in how the skull is represented.

Existing skull-modelling approaches span a broad spectrum of complexity. Homogeneous models represent the skull as a single layer with globally assigned density, sound speed, and attenuation (Aubry et al., 2022). Such models are attractive because they are simple and computationally efficient, whereas they neglect spatial heterogeneity and layer-specific structure. Layered models explicitly represent the outer and inner cortical tables and the intervening trabecular diploë, thereby capturing at least part of the skull's structural anisotropy. (Andrew Drainville et al., 2023; Angla et al., 2024). CT-derived models pursue greater realism by mapping HU information to spatially varying acoustic properties (Bancel et al., 2021b; Marsac et al., 2017a), including but not limited to density, sound speed, and frequency-dependent power-law absorption (Yaakub et al., 2023).

Despite their widespread adoption, these approaches have rarely been cross compared within a common experimental framework or benchmarked systematically against intracranial field measurement over a reasonably broad specimen set. This unresolved modelling uncertainty has direct implications for tFUS neuromodulation. A model may reproduce the gross beam pattern while still misestimating focal pressure, effective stimulated volume, or spatial targeting accuracy. Such errors are not trivial: inaccurate pressure prediction complicates insertion loss compensation, volumetric error affects the extent of exposed tissue, and focal displacement undermines confidence in target engagement. Systematic evaluation is therefore particularly important in low-intensity neuromodulation, where the intended intracranial effect is typically inferred from the planned acoustic field rather than confirmed directly during treatment.

Five skull-modelling strategies are therefore evaluated against hydrophone measurements acquired in ex-vivo human skull specimens. The chapter also includes a separate preliminary study in the rat, in which cone-coupled acoustic delivery is assessed experimentally by hydrophone mapping and combined with hippocampal electrophysiology.

6.2. Modelling framework

6.2.1. Skull specimens

Three historical calvaria (labelled S1-S3), two Thiel-embalmed calvaria (S4-S5; with scalp and dura retained) and two historical hemisected skulls (S6-S7) were used in this study. RoIs were defined as follows: three per calvarium over the frontal, left parietal, and right parietal bones, and two per half-skull over the parietal and temporal bones. RoI locations were chosen without optimisation for local morphology or target favourability, so that anatomically challenging regions, including areas near the frontal crest and squamous sutures, were intentionally retained. In total, 19 RoIs were analysed across the seven specimens. Figure 6-1 presents the corresponding thickness, bulk HU density, and SDR maps, together with the allocated source positions.

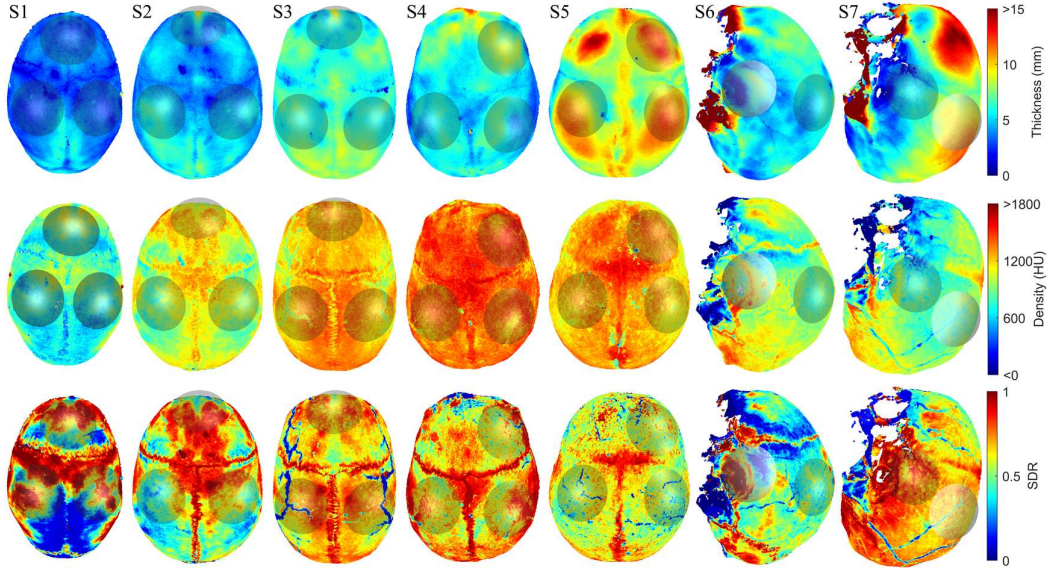


Figure 6-1 Skull specimens property maps, Upper row: Skull thickness maps of S1-S7. Middle row: the corresponding skull average HU density maps of S1-S7. Lower row: the corresponding SDR maps of S1-S7. Grey shades represent the RoI and the allocation of the acoustic source.

For each RoI, a sub-volume spanning $60 \text{ mm} \times 60 \text{ mm}$ at the outer skull surface was extracted and reoriented orthogonally for simulation. For the Thiel specimens, the attached soft tissue was neglected and only the bone was modelled.

6.2.2. Skull modelling strategies

To examine how skull modelling influences simulation fidelity, five skull-modelling strategies were implemented. In all cases, the simulated skull was described using mass density ρ (kg/m^3), sound speed c (m/s), and attenuation coefficient a ($\text{dB}/(\text{cm} \cdot \text{MHz}^2)$).

1. linear mapping with fixed attenuation (LM-Fa) assigns density and sound speed voxel-wise from CT while applying fixed attenuation coefficients (Krokhmal et al., 2025).

$$\begin{aligned}\rho_{skull} &= 0.66HU_{skull} + 1011, & HU_{skull} > 260 \\ c_{skull} &= 1.333\rho_{skull} + 166.7 \\ a_{skull} &= 13.3\end{aligned}$$

2. linear mapping with dynamic attenuation (LM-Da) also maps density and sound speed voxel-wise, but varies attenuation according to local porosity-related properties. (Yaakub et al., 2023):

$$\rho_{skull} = \frac{(\rho_{skull_max} - \rho_{water})HU_{skull}}{HU_{skull_max}} + \rho_{water}$$

$$c_{skull} = \frac{(c_{skull_max} - c_{water})(\rho_{skull} - \rho_{water})}{\rho_{skull_max} - \rho_{water}} + c_{water}$$

$$a_{skull} = (a_{skull_max} - a_{skull_min}) * (1 - \frac{HU_{skull} - HU_{skull_min}}{HU_{skull_max} - HU_{skull_min}})^{\frac{1}{2}} + a_{skull_min}$$

Where $\rho_{skull_max} = 1900$, $\rho_{water} = 1000$, $c_{skull_max} = 3100$, $c_{water} = 1500$ (all simulations), $a_{skull_max} = 18.1$, $a_{skull_min} = 1.9$.

3. fixed-property three-layer modelling (3L-F) segments the skull into outer cortical table, diploë, and inner cortical table, and assigns fixed acoustic properties to each layer.

$$\rho_{skull_cortical} = 1850, \rho_{skull_trabecular} = 1700 \quad (\text{Aubry et al., 2022})$$

$$c_{skull_cortical} = 2800, c_{skull_trabecular} = 2300 \quad (\text{Aubry et al., 2022})$$

$$a_{skull_cortical} = 4, \quad a_{skull_trabecular} = 16$$

4. dynamic three-layer modelling (3L-D) (see section 5.2.3).

$$\rho_{skull} = 0.66HU_{skull} + 1011, \quad \rho_{CT} > 260$$

$$c_{skull} = 0.70HU_{skull} + 1730$$

$$a_{skull_cortical} = 4, \quad a_{skull_trabecular} = 4 + 17(1 - SDR)$$

5. fixed-property single-layer modelling (1L-F) represents the skull as a homogeneous medium (Attali et al., 2023).

$$\rho_{skull} = 1860$$

$$c_{skull} = 2890$$

$$a_{skull} = 7.3$$

6.2.3. Experimental setup

To ensure rigid and repeatable transducer placement relative to each skull RoI, a helmet-shaped transducer-skull scaffold was designed individually for every RoI. The CT skull volume was first imported into 3D Slicer and segmented by thresholding. The skull mesh was then exported to MeshLab for surface repair and vertex reduction, and subsequently imported into SolidWorks. The outer skull surface was inverted to generate the inner surface of a conformal scaffold, and a clip mount was integrated to hold the transducer with a fixed 10 mm offset between the housing and the RoI surface (Figure 6-2.a). The final scaffold was then 3D printed (Figure 6-2.b). This scaffold-based strategy was considered more practical than external optical tracking, because the transducer was intended to remain fixed at a pre-defined location rather than be adjusted dynamically during the experiment.

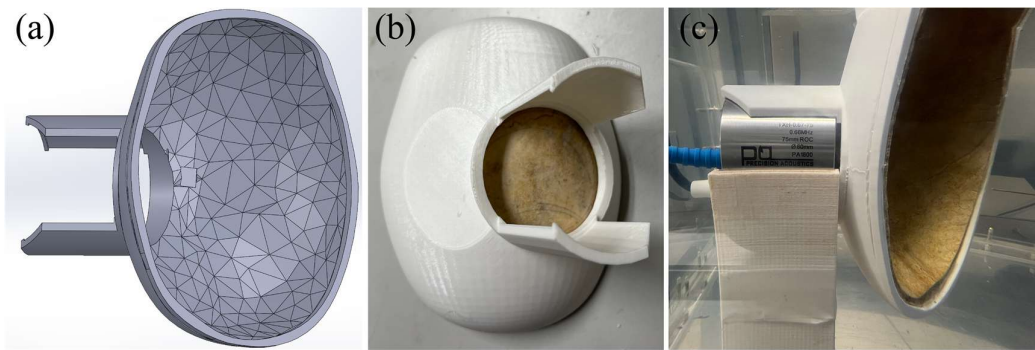


Figure 6-2 A representative transducer-skull fixture design: (a): Scaffold mesh model tightly compatible with skull outer surface with transducer fixture design (b): Integration of the skull with the scaffold, and with an open window for ultrasound transmission through the RoI. (c): Underwater integration of transducer-skull scaffold, the scaffold was mounted on the scaffold holder fixed on the water tank. Hydrophone was scanning at the right side, intracranial space.

For underwater measurements, a dedicated mounting frame was also constructed to preserve the relative placement of the transducer under both free-field and skull-present conditions. Free-field mapping was performed at the far-field focal depths of the three sources, namely 63 mm for 220 kHz, 75 mm for 680 kHz, and 74 mm for 1000 kHz. At each depth, raster scans were acquired over 30 mm $\times$ 30 mm for 220 kHz and 20 mm $\times$ 20 mm for 680 kHz and 1000 kHz. The same mapping geometry and depth were then repeated with the skull in place to enable direct comparison of insertion effects.

6.2.4. Evaluation metrics

To analyse focal behaviour away from the measurement plane, each measured lateral complex pressure map was zero-padded to an effective lateral extent of 40 mm and then propagated using *angularSpectrumCW*. Bidirectional propagation over a total axial range of 60 mm, from +45 mm to +105 mm, was used to estimate focal-volume variation and to locate the three-dimensional peak pressure within the evaluation domain.

For numerical prediction of the intracranial field, the measured source surface pressure was used directly as the source input. Each skull RoI segment was up-sampled to an isotropic grid spacing of 0.25 mm and parameterised using the five skull-modelling techniques described above. The skull RoI was placed 10 mm in front of the source. The simulation domain comprised $512 \times 288 \times 288$ voxels, corresponding approximately to lateral extents of -30 mm to +30 mm and an axial extent of 0 mm to 120 mm. This domain was chosen to encompass both the skull and the full RoI over which focal behaviour was analysed.

Simulation and measurement were compared using four complementary metrics: normalised peak pressure loss (p), focal intensity loss (I) at the free-field (*free*) and intracranial space (*IC*) far-field focal depth, focal-volume variation (V) over the propagated acoustic volume, and spatial peak displacement (D). These metrics were designed to assess not only whether the models reproduced insertion loss, but also whether they reproduced the shape and location of the intracranial focus. Peak-pressure and intensity metrics quantify energy transmission at the focal plane, focal-volume variation quantifies the extent of tissue exposed above a given threshold, and peak displacement quantifies spatial targeting fidelity. The corresponding equations to evaluate errors are shown below:

$$1. \text{ Insertion loss errors: } \varepsilon_p = \frac{|p_{sim} - p_{mea}|}{p_{mea}} * 100\%, \quad \varepsilon_I = \frac{|I_{sim} - I_{mea}|}{I_{mea}} * 100\%,$$

where p and I are insertion-normalised pressure: $p = \frac{p_{IC}}{p_{free}}$ and intensity: $I = \frac{I_{IC}}{I_{free}}$.

Intensity on the mapping plane: $I = \frac{1}{A_{-6dB}} \int_{A_{-6dB}} \frac{p^2}{\rho c} dA$, where A_{-6dB} is the -6dB coverage of the local peak.

$$2. \text{ Focal volume variation: } \varepsilon_{V,\tau} = \frac{|(V_\tau)_{sim} - (V_\tau)_{mea}|}{(V_\tau)_{mea}} * 100\%, \quad \tau \in \{-3, -6, -12 \text{ dB}\},$$

where $V\tau$ is insertion-normalised volume: $V\tau = \frac{(V\tau)_{IC}}{(V\tau)_{free}}$.

3. The peak location displacement $\varepsilon_D = |(\Delta D)_{sim} - (\Delta D)_{mea}|$,

where D is the vector distance from the free-field peak to the intracranial peak: $D = x_{IC} - x_{freefield}$, and ΔD is the signed axial-lateral displacement: $\Delta D = \text{sgn}(D_{axial})\sqrt{D_{axial}^2 + D_{lateral}^2}$.

6.3. Model performance

6.3.1. Representative case study

A representative example is shown in Figure 6-3 for a RoI located in the frontal bone of specimen S2 and modelled using the 3L-D strategy. Distortion was evident at all three frequencies. In each case, the intracranial focus was shallower than the free-field focus (indicated by black dot), and significant wave interactions occurred both in the transducer-skull spacing and within the skull thickness, at 220 kHz, the pressure in the gap region exceeded the intracranial focal pressure, indicating strong low-frequency superposition. At 680 kHz and 1000 kHz, clear sidelobes appeared, suggesting beam splitting associated with the frontal crest.

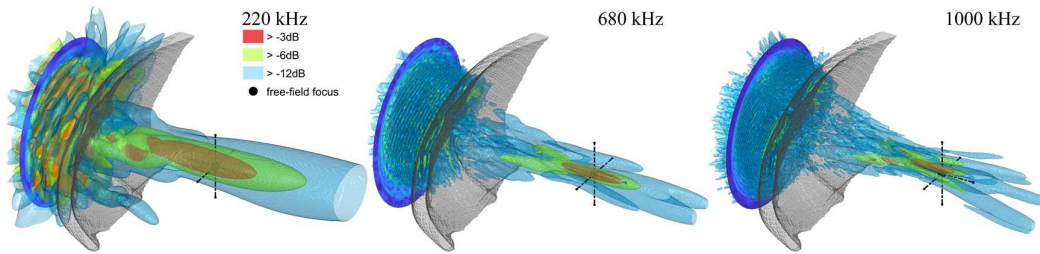


Figure 6-3 Simulated focal field with a piece of RoI at S2 frontal bone at 220 kHz, 680 kHz and 1000 kHz. Skull was modelled using 3L-D strategy. The pressure levels are normalised to peak intracranial field, black dot represent the focus at free-field.

Figure 6-4 compares the five skull-modelling strategies for the same RoI. Although the models produce markedly different distributions of properties, their intracranial focal-

pressure fields remained broadly similar in qualitative form. More substantial divergence emerges at 680 kHz and 1000 kHz, where the 3L-F, 3L-D, and 1L-F models reproduce enlarged sub-focal lobes, whereas the two linear-mapping models yield more compact foci.

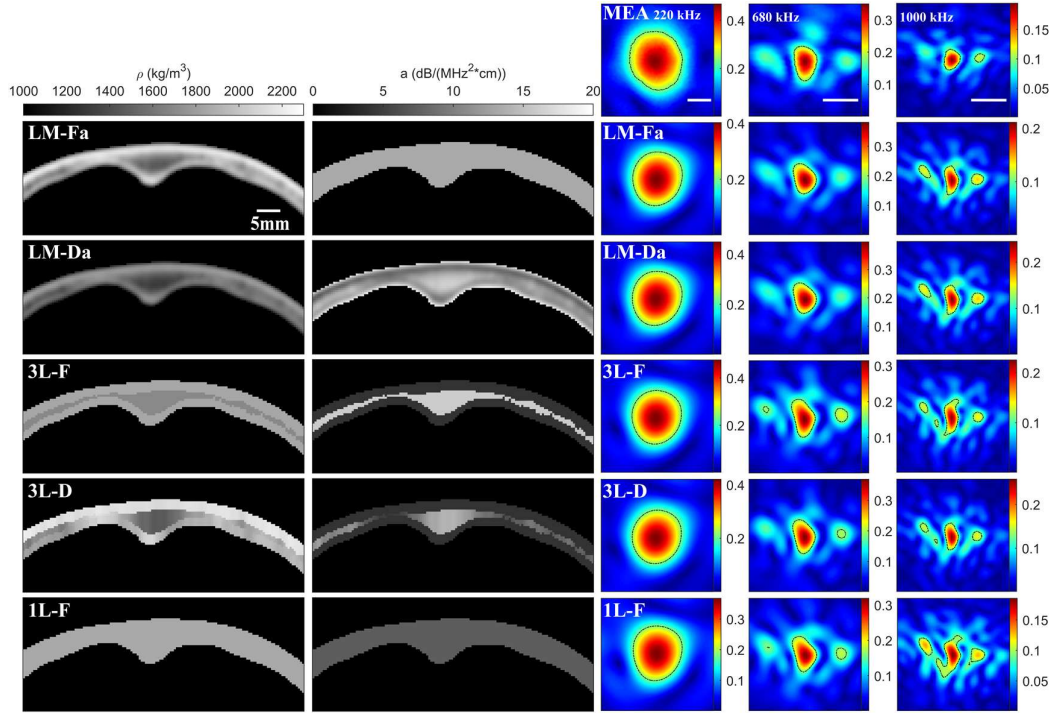


Figure 6-4 Outputs from the five models of a representative RoI at S2 frontal bone and its corresponding simulated cross-sectional focal field at 220 kHz, 680 kHz and 1000 kHz, compared with the corresponding measured fields.

Quantitatively, no single model achieves uniform superiority across all three frequencies, the relative peak pressure errors extended between +1.73% (3L-F) to -21.5% (1L-F), +13.8% (LM-Da) to -10.0% (3L-F), and +32.5% (3L-D) to -4.8% (1L-F) at the three frequencies, respectively. These data demonstrate the pronounced frequency dependence in modelling performance and suggest that the balance between anatomical fidelity and computational accuracy is likely to differ between low and high frequency tFUS applications.

6.3.2. Grouped field comparison

The 19 RoIs sampled a broad anatomical range: skull thickness varied from 3.1 mm (S1) to 11.2 mm (S5) (grouped mean $\pm$ std = 6.3 $\pm$ 2.3 mm); bulk HU density from 781

HU (S1) to 1445 HU (S5) (mean = 1092 ± 213 HU); and bulk SDR values between 0.39 (S6) to 0.73 (S1) (mean = 0.58 ± 0.08). Grouped median results are summarised in Figure 6-5 to Figure 6-8, and Table 6-1.

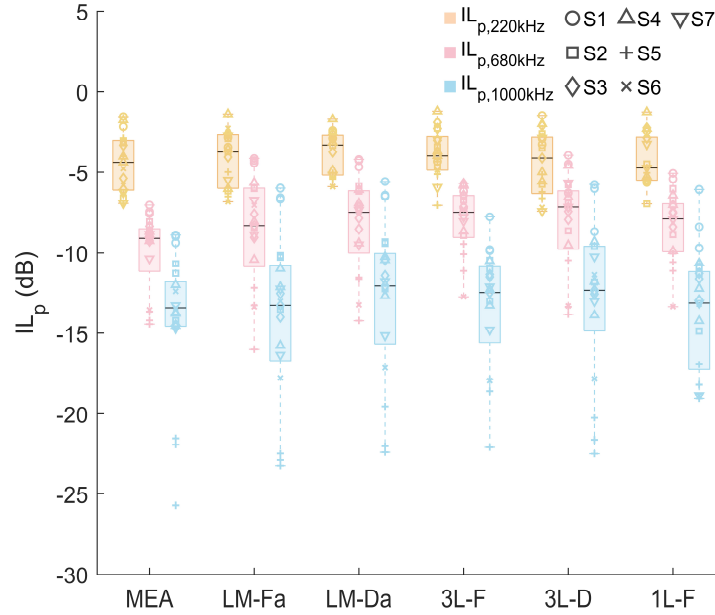


Figure 6-5 Measured (MEA) and simulated intracranial peak pressure loss across 19 RoIs from seven skull specimens at 220 kHz, 680 kHz and 1000 kHz frequencies. The results are shown in median values and 25% and 75% interquartile range, with whiskers extending to beyond the 1.5 interquartile range.

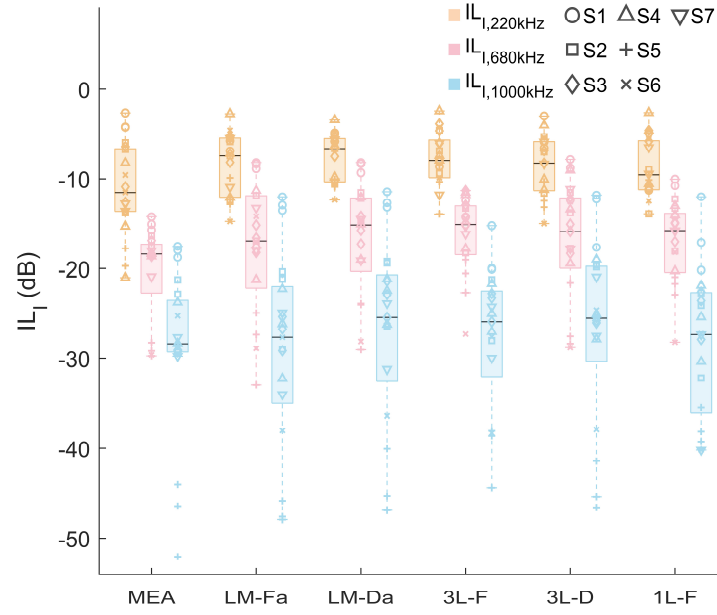


Figure 6-6 Measured (MEA) and simulated intracranial intensity (over -6dB cross-sectional beam area) loss across 19 RoIs from seven skull specimens at 220 kHz, 680 kHz and 1000 kHz frequencies.

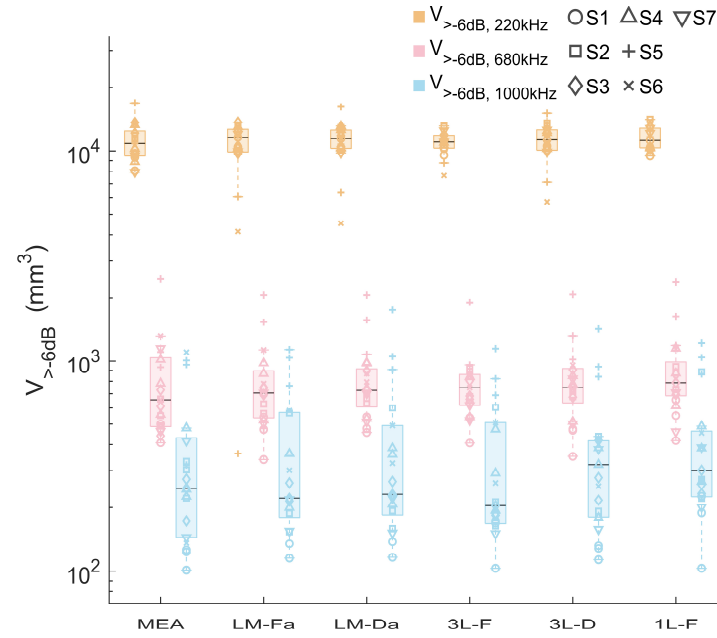


Figure 6-7 Hologram propagated from 2D measurement and simulated intracranial acoustic volumetric coverage (over -6dB) across 19 RoIs from seven skull specimens at 220 kHz, 680 kHz and 1000 kHz frequencies.

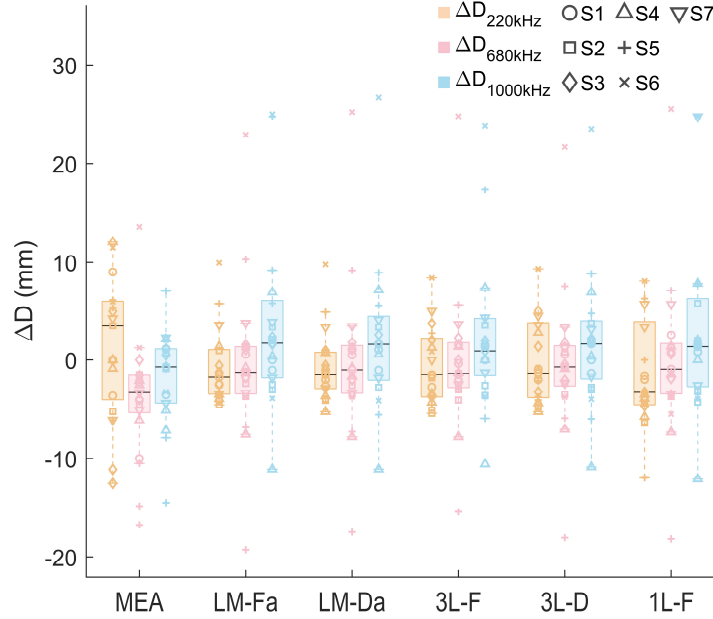


Figure 6-8 Hologram propagated from 2D measurement, and simulated intracranial peak pressure distance deviation to their free-field focus across 19 RoIs from seven skull specimens at 220 kHz, 680 kHz and 1000 kHz frequencies.

Compared with measurement, the simulated normalised peak-pressure loss (Figure 6-5) at the free-field focal depth differed by median values from -0.3 to +2.0 dB, across the three frequencies. The corresponding intensity loss (Figure 6-6) difference ranged from +0.8 to +4.9 dB. These systematically smaller simulated losses indicate that the current skull modelling strategies tended, on average, to underestimate attenuation or overestimate transmitted energy. This bias is more consequential for intensity than for pressure, because intensity depends on the square of pressure and is further modulated by beam area.

The absolute focal-volume discrepancy (Figure 6-7) above the -6 dB threshold decreased with increasing frequency: at 220 kHz the median discrepancy remained on the order of several hundred cubic millimetres, at 680 kHz it fell to the low hundreds, and at 1000 kHz it was typically only a few dozen cubic millimetres. However, because the measured focal volumes themselves became progressively smaller with increasing frequency, even modest absolute discrepancies represented a larger proportion of the measured volume. Accordingly, maximum relative discrepancy increased from 6.9% at 220 kHz to 29.0% at 1000 kHz. Thus, absolute focal-volume error diminished with frequency, but relative uncertainty remained substantial.

The simulated pressure maxima are on average located superficial to the measured peaks at 220 kHz, whereas the relationship reversed at 680 kHz and 1000 kHz (Figure 6-8). Despite these systematic shifts, the fixed-plane pressure difference remained modest: the simulated intracranial peak at the free-field focal depth plane differs from the three-dimensional peak by median values of 0.4%, 1.1% and 1.9% at the three frequencies, respectively; the corresponding experimental deviations are 4.5%, 1.2%, and 3.3%. These small percentages indicate that, in most cases, the highest intracranial pressure remained close to the free-field focus despite skull-induced field distortion.

Table 6-1 Measured and simulated intracranial acoustic field results at three frequencies, reported as median and IQR (in []).

	MEA	LM-Fa	LM-Da	3L-F	3L-D	1L-F
220 kHz						
IL_p (dB)	-4.4 [-6.1 -3.0]	-3.7 [-6.0 -2.7]	-3.3 [-5.2 -2.7]	-4.0 [-4.8 -2.8]	-4.1 [-6.3 -2.8]	-4.7 [-5.5 -2.8]
IL_I (dB)	-11.5 [-13.7 -6.7]	-7.5 [-12.1 -5.5]	-6.6 [-10.3 -5.5]	-8.0 [-9.9 -5.7]	-8.3 [-11.3 -5.8]	-9.6 [-11.2 -5.7]
V_{-6dB} (mm ³)	10827 [9468 12460]	11573 [9885 12701]	11413 [10275 12588]	11004 [10300 11852]	11261 [10072 12619]	11179 [10337 12860]
ΔD (mm)	3.5 [-4.0 6.0]	-1.8 [-3.5 1.0]	-1.5 [-2.9 0.8]	-1.5 [-3.8 2.2]	-1.3 [-3.8 3.9]	-3.3 [-4.6 4.0]
680 kHz						
IL_p (dB)	-9.1 [-11.2 -8.5]	-8.3 [-10.8 -6.0]	-7.5 [-10.0 -6.1]	-7.5 [-9.1 -6.4]	-7.1 [-9.8 -6.2]	-7.9 [-9.9 -6.9]
IL_I (dB)	-18.4 [-22.7 -17.3]	-16.9 [-22.2 -11.9]	-15.2 [-20.3 -12.1]	-15.1 [-18.4 -13.0]	-15.9 [-20.0 -12.1]	-15.7 [-20.4 -13.9]
V_{-6dB} (mm ³)	655 [487 1040]	705 [536 896]	725 [608 916]	746 [617 861]	747 [634 919]	788 [684 990]
ΔD (mm)	-3.3 [-5.3 -1.6]	-1.3 [-3.5 1.3]	-1.0 [-3.4 1.5]	-1.4 [-2.8 1.7]	-0.7 [-2.7 1.4]	-0.9 [-3.4 1.7]
1000 kHz						
IL_p (dB)	-13.4 [-14.6 -11.8]	-13.3 [-16.7 -10.8]	-12.1 [-15.6 -10.0]	-12.5 [-15.6 -10.9]	-12.3 [-14.9 -9.6]	-13.1 [-17.2 -11.2]
IL_I (dB)	-28.4 [-29.3 -23.5]	-27.6 [-35.0 -22.0]	-25.5 [-32.5 -20.7]	-25.9 [-32.0 -22.5]	-25.5 [-30.3 -19.8]	-27.3 [-36.1 -22.7]
V_{-6dB} (mm ³)	248 [145 431]	222 [180 570]	232 [185 492]	204 [168 508]	320 [181 418]	301 [226 464]
ΔD (mm)	-0.7 [-4.4 1.1]	1.7 [-1.8 6.1]	1.6 [-2.1 4.5]	0.9 [-1.6 4.3]	1.6 [-2.0 4.1]	1.3 [-2.7 6.3]

6.3.3. Model-specific error and specimen effects

Table 6-2 summarizes the averaged absolute errors obtained when the five skull modelling approaches are benchmarked against the 19 experimental intracranial datasets. Across all frequencies, ε_p clustered in the range of 20% (3L-D) to 31% (LM-Da), with frequency and modelling methods averaged values of $23 \pm 16\%$ at 220 kHz, $25 \pm 18\%$ at 680 kHz, and $27 \pm 25\%$ at 1000 kHz, respectively. The corresponding ε_I are in the range of 41% (LM-Fa) to 77% (1L-F), and giving frequency and modelling methods averaged errors of $60 \pm 51\%$, $63 \pm 52\%$, and $60 \pm 76\%$, respectively.

Table 6-2 Average intracranial errors of simulated results using 5 skull modelling methods compared to the measurement at 220 kHz, 680 kHz and 1000 kHz.

	LM-Fa	LM-Da	3L-F	3L-D	1L-F
220 kHz					
ε_p (%)	22 $\pm$ 17	21 $\pm$ 16	25 $\pm$ 19	20 $\pm$ 16	28 $\pm$ 14
ε_I (%)	54 $\pm$ 47	57 $\pm$ 53	65 $\pm$ 58	50 $\pm$ 47	72 $\pm$ 51
$\varepsilon_{V,-3dB}$ (%)	32 $\pm$ 32	31 $\pm$ 33	31 $\pm$ 40	32 $\pm$ 31	37 $\pm$ 59
$\varepsilon_{V,-6dB}$ (%)	25 $\pm$ 25	22 $\pm$ 18	18 $\pm$ 22	22 $\pm$ 13	20 $\pm$ 14
$\varepsilon_{V,-12dB}$ (%)	15 $\pm$ 10	15 $\pm$ 10	13 $\pm$ 8	14 $\pm$ 8	16 $\pm$ 11
ε_D (mm)	6.4 $\pm$ 3.9	6.3 $\pm$ 4.2	7.1 $\pm$ 4.6	6.7 $\pm$ 4.8	6.3 $\pm$ 4.3
680 kHz					
ε_p (%)	24 $\pm$ 17	25 $\pm$ 14	27 $\pm$ 21	27 $\pm$ 22	23 $\pm$ 17
ε_I (%)	59 $\pm$ 46	61 $\pm$ 41	72 $\pm$ 62	65 $\pm$ 67	57 $\pm$ 46
$\varepsilon_{V,-3dB}$ (%)	26 $\pm$ 20	25 $\pm$ 22	27 $\pm$ 22	25 $\pm$ 20	34 $\pm$ 27
$\varepsilon_{V,-6dB}$ (%)	25 $\pm$ 20	25 $\pm$ 20	27 $\pm$ 21	25 $\pm$ 22	36 $\pm$ 24
$\varepsilon_{V,-12dB}$ (%)	16 $\pm$ 18	14 $\pm$ 13	13 $\pm$ 10	17 $\pm$ 15	19 $\pm$ 11
ε_D (mm)	5.2 $\pm$ 4.9	5.1 $\pm$ 4.8	5.0 $\pm$ 4.7	5.1 $\pm$ 4.4	5.6 $\pm$ 4.9
1000 kHz					
ε_p (%)	22 $\pm$ 12	31 $\pm$ 16	26 $\pm$ 33	27 $\pm$ 19	30 $\pm$ 39
ε_I (%)	41 $\pm$ 28	67 $\pm$ 38	62 $\pm$ 92	56 $\pm$ 46	77 $\pm$ 129
$\varepsilon_{V,-3dB}$ (%)	40 $\pm$ 43	42 $\pm$ 50	43 $\pm$ 53	40 $\pm$ 43	56 $\pm$ 65
$\varepsilon_{V,-6dB}$ (%)	43 $\pm$ 38	51 $\pm$ 48	42 $\pm$ 41	49 $\pm$ 46	67 $\pm$ 83
$\varepsilon_{V,-12dB}$ (%)	25 $\pm$ 24	27 $\pm$ 25	28 $\pm$ 28	25 $\pm$ 30	39 $\pm$ 47
ε_D (mm)	6.2 $\pm$ 9.6	4.8 $\pm$ 6.0	6.0 $\pm$ 8.1	4.6 $\pm$ 5.3	6.3 $\pm$ 7.2

Among the five models, LM-Fa yielded peak-pressure errors consistently below the ensemble mean at all three frequencies. The 1L-F strategy performed worst at 220 kHz and 1000 kHz, whereas 3L-F was least accurate at 680 kHz. No single model dominated across all evaluation dimensions. This indicates that increasing anatomical complexity

does not automatically improve predictive accuracy if the mapped acoustic properties, particularly attenuation, are not sufficiently reliable.

Volumetric accuracy, assessed at the -3dB, -6dB and -12 dB thresholds, showed a similar pattern. Averaged across models, the volume errors were $33\pm39\%$, $21\pm17\%$, and $15\pm10\%$ at 220 kHz; $27\pm22\%$, $28\pm21\%$, and $16\pm14\%$ at 680 kHz; and $44\pm51\%$, $50\pm53\%$, and $29\pm32\%$ at 1000 kHz. Again, 1L-F tended to produce the largest deviations, whereas the remaining four approaches were broadly comparable.

Spatial fidelity, quantified by the signed axial-lateral displacement (ε_D), showed only modest differences among models. Mean displacement errors were 6.6 ± 4.3 mm at 220 kHz, 5.2 ± 4.7 mm at 680 kHz, and 5.6 ± 7.3 mm at 1000 kHz. LM-Da performed marginally better than the other models, but the advantage was less than 1 mm and therefore not practically decisive.

The two specimen cohorts also differed substantially in skull properties. Historical calvarial RoIs had mean thickness of 5.8 ± 1.7 mm, mean CT HU value of 987 ± 139 HU, and SDR of 0.54 ± 0.09 . whereas Thiel-embalmed RoIs were thicker and denser, with values of 8.5 ± 2.5 mm, 1359 ± 88 HU, and 0.62 ± 0.07 respectively. These differences were reflected in the larger energy-loss errors observed in the Thiel group, which is summarised in Table 6-3.

Table 6-3 Pressure and intensity errors in comparison to different sample conditions.

	Historical RoIs			Thiel RoIs		
	220 kHz	680 kHz	1000 kHz	220 kHz	680 kHz	1000 kHz
ε_p (%)	23 ± 18	22 ± 16	25 ± 16	24 ± 11	31 ± 22	32 ± 38
ε_I (%)	52 ± 46	54 ± 44	51 ± 40	77 ± 57	81 ± 64	81 ± 121

Another reasonable explanation is that the employed modelling strategies derived from skull with different preservation conditions. There is currently a lack of Thiel-embalmed skull acoustic-property data, for formalin-fixed human skull, the longitudinal attenuation coefficient is approximately at $8.83 \text{ dB}/(\text{cm}\cdot\text{MHz}^{1.43})$ (Andrew Drainville et al., 2023; Treeby et al., 2014; P. J. White et al., 2006b). Also, the embalmed material retains attached scalp and dura mater, media that were omitted from the simulations yet participate in the experimental transmission path; their combined

absorption and impedance mismatch would elevate the measured insertion loss relative to a bone-only model.

6.4. Discussion

A key finding is that skull modelling remains the dominant source of uncertainty in k-Wave-based tFUS simulation. Peak pressure errors remained on the order of 20 -31%, but intensity errors were substantially larger because they were amplified by the nonlinear dependence on pressure and by variation in beam width. Volumetric discrepancies at the -6 dB threshold were also substantial, especially at higher frequency when the focal volume became smaller and therefore more sensitive to modest modelling error.

In principle, the fully voxelised LM-Da approach might be expected to perform best, because it retains local heterogeneity in density, sound speed, and attenuation. However, the present data do not support that expectation. An alternative study was conducted by using a different set of attenuation coefficient: $a_{skull,max}=8.7$ dB/(cm·MHz^{1.43}), $a_{skull,min}=4$ dB/(cm·MHz^{1.43}) (Yaakub et al., 2023) at 680 kHz, the median deviations in peak pressure and intensity increased to +1.9 dB and +4.0 dB, respectively, exceeding those obtained with the parameter set adopted in Section 6.2.2. This suggests that uncertainty in attenuation assignment may outweigh the theoretical benefit of retaining fully voxel-wise heterogeneity. Notwithstanding, the present study did not isolate the effect of attenuation independently from the associated sound-speed and density transforms, so that conclusion remains provisional.

Blurred HU gradients at the air-bone boundary are also likely to render the outer and inner cortical tables artificially low in density, thereby exaggerating attenuation in the linear-mapping models. Reassignment of these transitional voxels to cortical-like properties may therefore improve predictive performance. This interpretation is supported by the good performance of LM-Fa. Despite its simplicity, LM-Fa produced the lowest average pressure error across the three frequencies. Conversely, the

homogeneous 1L-F model was generally the least accurate, indicating that some anatomical stratification is necessary even if full voxel-wise heterogeneity does not yet yield a clear advantage under the present parameter mappings. The marginal difference between 3L-F and 3L-D further suggests that model structure alone is not sufficient; the accuracy of the mapped acoustic properties is at least as important as the geometric fidelity of the skull representation.

Comparison with a benchmark 3L-F configuration also supports this conclusion (Aubry et al., 2022). In the benchmark setup, attenuation coefficients equivalent to 16 and 32 dB/(cm·MHz²) for cortical and trabecular bone. The benchmark setup produced peak pressure levels that were lower than measurement by -0.9 dB ($\epsilon_p=23\pm19\%$), -1.6 dB ($20\pm15\%$), and -5.2 dB ($46\pm19\%$) at 220 kHz, 680 kHz and 1000 kHz, respectively. Although this overestimation of attenuation can reduce error at lower frequency, it produces substantially larger error at 1000 kHz and would risk overshoot if used to tune transducer input energy.

It is worth noting that two methodological constraints exacerbate these uncertainties. First, the lateral mapping window (20mm-30mm) only spanning over twice the focal width, this is due to a constrained time frame for collecting experimental data; a larger aperture is empirically required to capture the full energy distribution and to yield reliable reconstructions. Second, acquiring holograms exactly at the focal plane may sub-optimal as the energy is tightly localized, sampling at a slightly defocused plane where the lateral spread is broader could preserve more phase information, albeit at the cost of additional acquisition time.

The most important practical implication of this study is that current skull-modelling strategies are sufficient to reproduce the gross beam pattern, but not yet sufficiently reliable for fully dose-controlled neuromodulation planning. Even when the focal location is approximately correct, uncertainty in intensity and focal-volume prediction remains large. In neuromodulation, this is non-trivial: inaccurate intensity estimation weakens the dose-response relationship, impairs cross-subject normalisation, and complicates safety margins intended to avoid unwanted thermal or mechanical effects. Likewise, focal-volume error changes the extent of tissue exposed above stimulation-relevant thresholds.

Simulation-guided planning should therefore be interpreted, at present, as a tool for comparative assessment and hypothesis testing rather than as a definitive predictor of delivered intracranial dose. More realistic physical representation of simulation domain will be required before simulation-guided transcranial ultrasound can approach the reliability needed for quantitatively controlled use.

6.5. In-vivo evaluation

Having established source behaviour, transcranial transmission characteristics, analytical insertion loss prediction, and modelling uncertainty in the preceding chapters, a further practical question is whether a tFUS system can be combined with in-vivo electrophysiology while preserving interpretable recordings. Among candidate targets, the hippocampus is of particular interest because of its central role in memory and cognition. In humans, intracranial recordings show that a subset of hippocampal neurons exhibits episode-specific reinstatement across encoding and retrieval (Kolibius et al., 2023), while rodent studies support the idea that hippocampal processing can generalise by recombining previously learned representational structures to support rapid adaptation to new situations without starting from scratch (Bakermans et al., 2025).

At a mechanistic level, memory is increasingly viewed as being implemented by distributed neural ensembles, with synaptic plasticity serving as a major substrate for forming and stabilising those ensembles (Abraham et al., 2019). Anterograde manipulations that disrupt plasticity mechanisms can impair hippocampus-dependent learning (S. J. Martin et al., 2000). This motivates the hypothesis that interventions capable of promoting or reinstating learning-related synaptic weight changes could support memory restoration (Neves et al., 2008). At the same time, establishing causal links between physiology and memory likely requires assays sensitive to learning-engaged ensembles and replay-related activity patterns, rather than relying exclusively on global plasticity readouts (Kolibius et al., 2023).

In hippocampal electrophysiology, classic high-frequency electrical tetanisation can induce long-term potentiation (LTP) and produce lasting increases in evoked synaptic responses (Gonzalez et al., 2013). More recently, ultrasound-based perturbation has also been reported to influence hippocampal circuit function. Notably, brief theta-pattern tFUS applied to hippocampus has been reported to produce lasting enhancement of basal synaptic transmission and facilitated the LTP (Watts et al., 2025), whereas tFUS modulations in hippocampal circuit can produce depression (Niu et al., 2022). Plausibly, hippocampal networks are sensitive to ultrasound, and that the physiological consequences of tFUS depend strongly on stimulation parameters and experimental context.

This section therefore presents a preliminary rat experiment combining tFUS with perforant path–dentate gyrus (pp–DG) electrophysiology. In addition to evaluating ex-vivo acoustic delivery through the rat skull, it tests whether hippocampal synaptic input, indexed by the pp–DG field excitatory postsynaptic potential (fEPSP), and neuronal output, indexed by the dentate population spike (PS), can be recorded stably during tFUS. Because oscillatory network activity may provide an additional view of hippocampal excitability, LFP spectral features were also quantified descriptively. The central aim of this section is to establish an experimental platform, evaluate its acoustic and recording feasibility, and define the methodological requirements for future controlled studies.

6.5.1. Rat intracranial acoustic characterisation

A male rat skull was employed to validate the acoustic delivery parameter for in-vivo tFUS. A water-filled coupling cone (Precision Acoustics, UK; tip diameter 20 mm; outlet diameter 14.5 mm) was mounted to the 650 kHz FUS transducer. The cone was filled with degassed water and sealed at the tip using a transparent membrane with greater than 99% transmission. Care was taken to remove visible air bubbles introduced during filling.

To characterise the free-field output with the coupling cone in place, the acoustic field was mapped using a 1 mm hydrophone positioned 3 mm from the cone window. Relative to the transducer without the cone, focal intensity remained at 95% of the

original value, the lateral FWHM was 4.1 mm compared with 4.2 mm without the cone, and the axial depth of the half-maximum region was 15.5 mm (Figure 6-9.a).

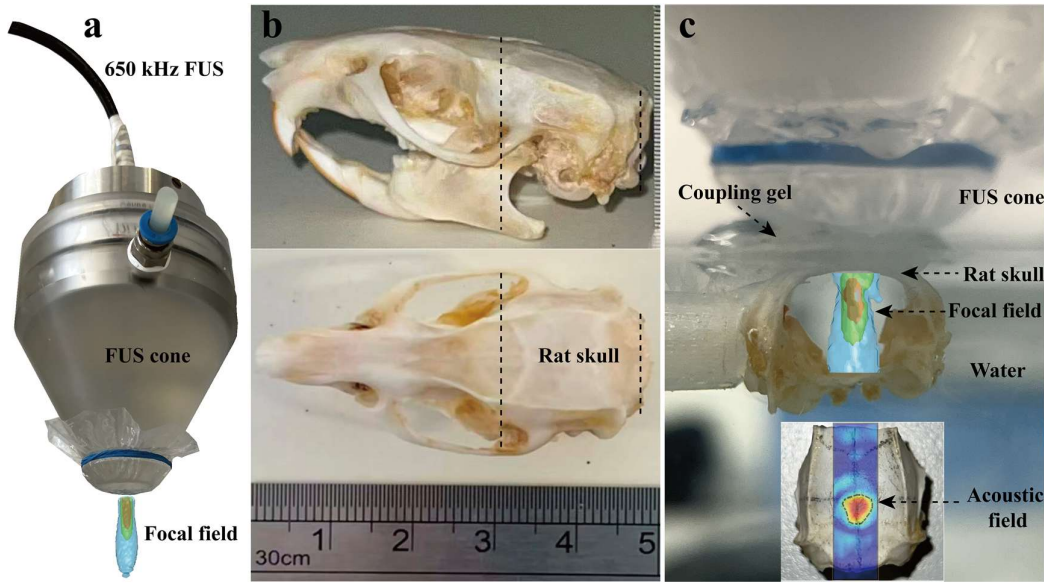


Figure 6-9 (a): 650 kHz FUS transducer integrated with cone, cone is filled with degassed water; and its 3D acoustic focal field. (b): representative rat skull. The frontal bone and the occipital bone were removed at the dashed lines to allow the access of the hydrophone. (c): the intracranial acoustic field experiment. The skull was immersed just below the water surface, the cone and skull were coupled with ultrasound gel. The hydrophone was inserted horizontally in the skull cavity, and showed the 2D acoustic field and 3D focal field.

To evaluate the intracranial acoustic characteristics, the frontal and occipital bones were removed so that the cranial cavity remained largely intact while still allowing hydrophone manoeuvring (Figure 6-9.b). The skull was positioned such that it remained immersed just below the water surface, and the cone outlet was coupled to the dorsal skull surface via a layer of ultrasound gel. A needle hydrophone was inserted horizontally within the cranial cavity and scanned over a $6 \text{ mm} \times 20 \text{ mm}$ region, yielding 2D and reconstructed 3D representations of the intracranial acoustic field (Figure 6-9.c).

Compared with the conventional mapping arrangement in which the hydrophone sensing element is oriented orthogonal to the direction of wave propagation, the present configuration placed the sensing element nearly parallel to the wave direction. This reduced direct hydrophone excitation and therefore decreased received signal amplitude

by approximately 44%, although the overall field profile remained comparable to that observed in a conventional arrangement.

With the skull in place, the received signal amplitude and focal intensity were attenuated by 18.5% and 37.8%, respectively. The lateral FWHM and axial half-maximum depth were 3.1 mm and 8.5 mm, respectively. The estimated -3dB focal volume was 17.8 mm³, with the focus centred approximately 2.5 mm below the skull inner surface. This focal depth was considered relevant for hippocampal targeting because cornu ammonis (CA) subfields and DG are typically located at approximately 3 mm below the skull surface in the rat (Aksić et al., 2013).

6.5.2. Electrophysiology and stimulation configuration

A single male Lister-hooded rat (N=1; body weight 400 g; age 5-6 weeks) was used in this feasibility study. All procedures were subjected to local ethical review at the University of Dundee and performed by a licensed operator.

Anaesthesia was induced with 1.9 ml of urethane (1.5g/kg) and maintained with supplementary 0.2 ml injection as required. Depth of anaesthesia was assessed periodically by toe pinch, and body temperature was maintained at 36.2°C. The scalp was shaved and the rat was positioned in a stereotaxic frame with ear bar fixture. After exposing the skull, bregma was identified and used as the stereotaxic reference point for anteroposterior (A-P) and mediolateral (ML) coordinates. Saline was applied to exposed tissue to reduce drying during the experiment.

Electrical stimulation and recording targeted the pp and DG, respectively (Figure 6-10.c and d). Coordinates based on the Paxinos atlas were A-P = -7.5 mm, ML = -4.0 mm (pp), and A-P = -3.5 mm, ML = -2.0 mm (DG) (Figure 6-10.b), with approximate dorsal-ventral (DV) targets of -2.5 mm and -3.0 mm, respectively. Burr holes were drilled at the designated sites to permit electrode insertion.

To accommodate the FUS hardware, the stimulation and recording electrodes (polytetrafluoroethylene-insulated platinum/iridium) were shaped to clear the FUS cone while allowing vertical descent into the target (Figure 6-10.a). Electrodes were sleeved in cannula to increase rigidity and allow controlled lowering to the intended

depths. All recordings were performed on a workbench enclosed, apart from the access site, within a Faraday cage, and electrical components were grounded to minimize environmental electrical noise.

Electrical stimulation was delivered using brief square pulses at 0.05 Hz by Neurolog system (NL800A; Digitimer Ltd., Herts., UK). Stimulation intensity was set at 500 μ A, which empirically produced a stable fEPSP response while avoiding saturation observed above approximately 1 mA. To localize the recording site accurately, the stimulation electrode was first placed at the predetermined depth, the recording electrode was then lowered into the DG in stepwise increments until a characteristic fEPSP waveform was observed (Aksoy-Aksel & Manahan-Vaughan, 2013), the electrical stimulation amplitude was kept constant and recordings were continued until fEPSP responses stabilised. The fEPSPs and LFP were digitised at 20 kHz and band-pass filtered between 1 Hz and 5 kHz using in-house built LabVIEW software.

PS amplitude was used to evaluate the neuronal output and was quantified as the vertical distance between the most negative deflection and the straight line connecting the first and second positive peaks of the evoked waveform. This metric was chosen because the PS reflects synchronous discharge of a local neuronal population and therefore complements the fEPSP slope, which primarily indexes synaptic input.

For spectral analysis, LFP segments following each electrical stimulation were extracted after exclusion of the stimulation artefacts window. Power spectral density (PSD, μ V²) was evaluated from 0.1 Hz to 10 Hz using the Welch method with 0.1 Hz frequency resolution. All processing and analysis were performed in MATLAB.

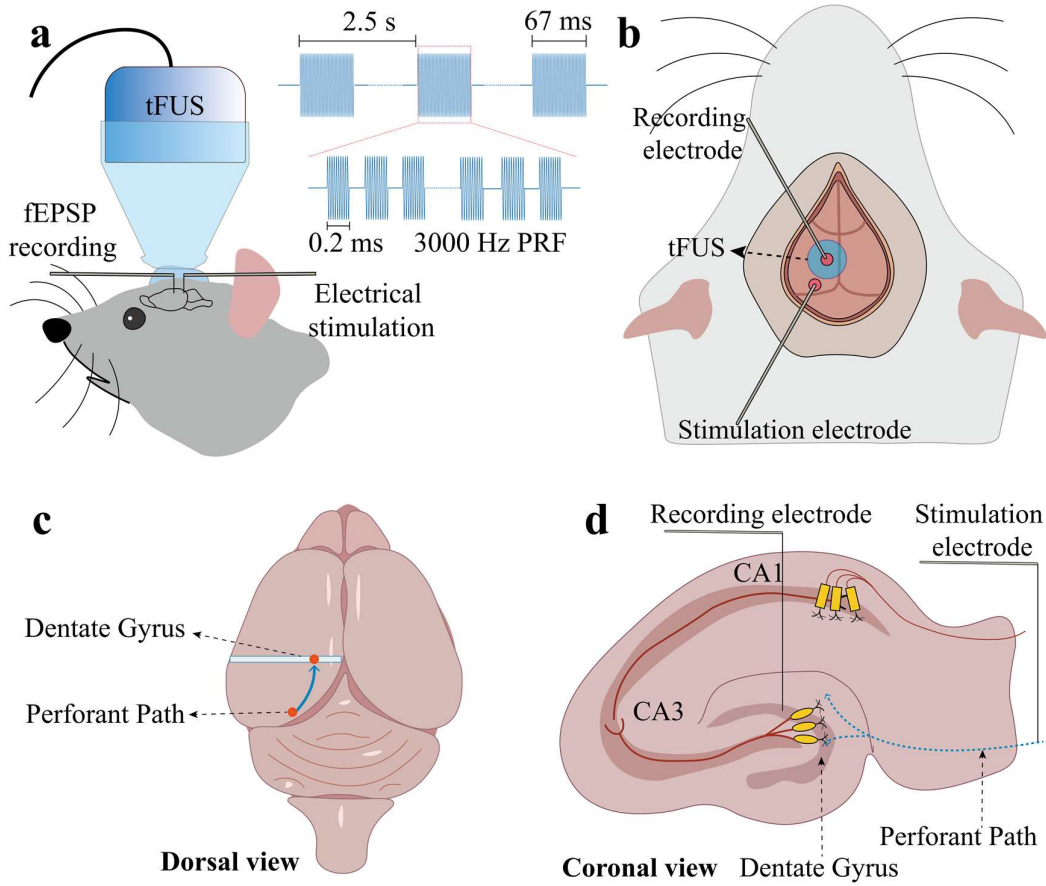


Figure 6-10 (a): schematic stimulation experimental setup and the delivered US waveform. (b): the placement of stimulation and recording electrodes. (c): The representative stimulation and recording brain targets in the dorsal view. (d) the coronal view of the recording site of the DG. The graphs were hand drawn using Adobe Illustrator.

tFUS was delivered using a 650 kHz carrier with a pulsed waveform consistent with recent publications (Ramachandran et al., 2025; Yu et al., 2021). Each sonication had a PD of 67 ms and an inter-sonication interval of 2.5 s. Within each 67 ms sonication, ultrasound was delivered as a train of 0.2 ms tone bursts at a PRF of 3000 Hz, corresponding to an approximate DC of 60% (Figure 6-10.a).

During stimulation, the cone outlet was positioned above the recording site and coupled to the skull using ultrasound gel to optimize transmission. The acoustic input power was adjusted according to the transmission losses measured in the ex-vivo rat skull characterisation. The predicted in-situ peak pressure and spatial-peak pulse-average intensity (I_{SPPA}) were estimated as 360 kPa and 4.4 W/cm², respectively.

The experimental session lasted 17 mins and comprised 8 mins with the tFUS system positioned but not firing (baseline), 5 mins of active tFUS sonication, and 4 mins post-sonication. In total, 51 pp-DG recordings were acquired from this single animal.

6.5.3. Preliminary physiological observations

Across the session, hippocampal evoked responses remained measurable during active tFUS exposure. Visual inspection of the waveforms indicated that the overall morphology of the pp-DG response was preserved during sonication. This suggests that the coupling-cone setup enabled acoustic delivery without overt disruption of hippocampal electrophysiological recording quality. However, continuous LFP monitoring revealed a brief sonication-related artefact during active stimulation. To preserve analysis quality, 0.5 s of raw signal containing the artefact was excluded from the active-sonication epoch before spectral analysis.

Figure 6-11.a shows the averaged pp-DG evoked responses during baseline (namely Control) and active sonication. Across epochs, the overall waveform shape remained stable, including the latency and form of the early synaptic component and the superimposed PS. The mean baseline fEPSP slope, estimated by linear fitting between two fixed time points, was 2.90 mV/ms.

The average PS amplitude exhibited a small increase during sonication. Relative to the baseline value of 4.95 mV, PS amplitude increased by 4.4% during active tFUS and decreased by 2.9% in the post-tFUS period (Figure 6-11.b). Although the direction of change suggests a small increase in DG population spiking during sonication, the magnitude was modest and should be interpreted cautiously in light of the single-session design and potential confounds such as slow drift in excitability.

Notwithstanding, these observations suggest that tFUS may have produced a subtle modulation of neuronal output, while the synaptic input component remained comparatively stable under the present stimulation conditions.

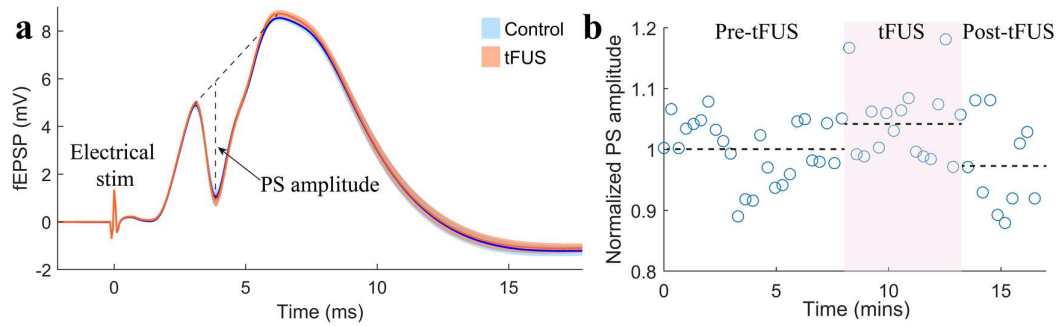


Figure 6-11.(a): Average pp-DG fEPSP waveform during baseline (control) and active tFUS stimulation. Shading indicates waveform variability across trials within each epoch. Dashed lines show the PS amplitude definition. (b): PS amplitudes across pre-tFUS, during tFUS and post-tFUS epochs, results were normalised to the pre-tFUS mean.

Representative LFP segments from each epoch are shown in Figure 6-12.a. While overall signal amplitude was broadly comparable across the session, spectral analysis suggested an increase in low-theta rhythmicity during active sonication (Figure 6-12.b).

PSD showed a dominant low-frequency peak at approximately 3.8-4.0 Hz in all epochs. Relative to the pre-tFUS baseline, the peak PSD near 3.8-4.0 Hz increased by 24.8% during active sonication and decreased by 20.6% post sonication.

Band-limited changes showed a similar qualitative pattern. During tFUS, PSD increased by 7.5% in the 0.1-3.0 Hz band, 10.2% in the 3.1-6.0 Hz band, and 13.2% in the 6.1-9.0 Hz band. In post-tFUS epoch, the corresponding changes were 11.0%, 3.1%, and -0.5%, respectively. These descriptive observations are consistent with a transient enhancement of low-frequency hippocampal power during active sonication, followed by partial return toward baseline after cessation.

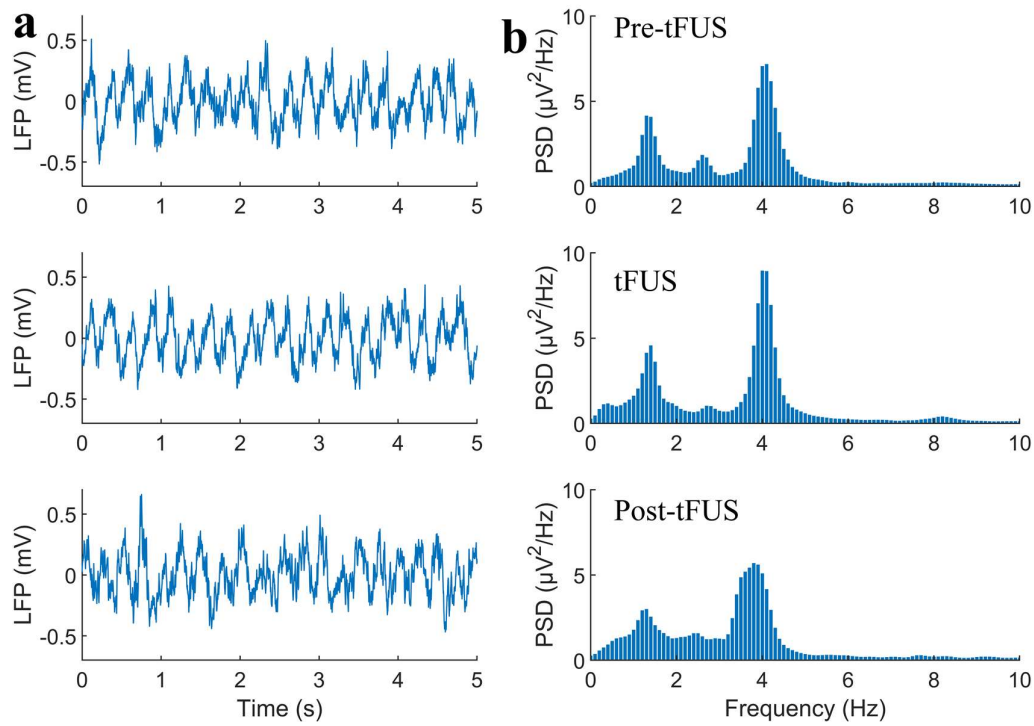


Figure 6-12 (a): Representative LFP segments from pre, during and post tFUS epochs. (b): power spectral density (PSD) for each epoch.

However, given the absence of a sham sonication and the single-session design, these spectral changes cannot be attributed uniquely to ultrasound. Nonetheless, the key feasibility finding is that meaningful LFP spectral features were preserved and quantifiable during coupling-cone tFUS delivery.

6.5.4. In-vivo feasibility and limitations

Intracranial mapping quantified both attenuation and focal geometry and therefore supports the basic feasibility of delivering a reasonably focal field into the targeted brain region. At the same time, it is important to note that in-situ dose remains sensitive to alignment and coupling conditions, and skull-to-skull variability can potentially be substantial. Consequently, while the mapping supports plausible hippocampal engagement, uncertainty in the exact dose delivered to the intended subfield limits

confidence in attributing the observed physiological effects specifically to that subfield rather than to adjacent hippocampal tissue.

A central feasibility outcome of this study is that cone-coupled tFUS could be delivered while preserving measurable evoked responses. Within the tested protocol, the fEPSP slope remained stable, suggesting that the early synaptic component of the pp-DG response was not materially altered under the present conditions. Spectral analysis suggested a possible transient enhancement of low-frequency activity during active sonication.

Several limitations follow directly from the feasibility nature of this work. First, the absence of a sham-sonication condition limits causal interpretation, particularly for the LFP results. The baseline period controlled for transducer placement, but did not control for auditory or mechanical effects associated with driving the transducer. A future sham should therefore include either blocked acoustic transmission or an off-target matched sonication condition. Second, although stereotaxic coordinates and electrophysiological signatures were used to guide targeting, no post hoc anatomical verification was reported. Histological verification or electrode marking would improve confidence in both the electrophysiological placement and the relation of the acoustic focus to the intended hippocampal subfields. Third, the post-sonication monitoring period was short. Because plasticity-like aftereffects are of particular interest in hippocampal neuromodulation, longer post-stimulation recording periods will be necessary to determine whether any persistent synaptic or oscillatory changes emerge. Finally, the study was restricted to a single successful in-vivo session, in part because of disruption caused by institutional transition during the project. This necessarily limits the scope of interpretation, nevertheless, it does not diminish the value of the study as a platform-validation exercise.

7. Conclusion

This final chapter draws together the main findings of the thesis, considers their broader significance for tFUS neuromodulation, and outlines several directions for future work.

7.1. Summary

This thesis addressed a central methodological problem in tFUS neuromodulation: how to move from nominal source settings to a physically interpretable estimate of the acoustic field delivered through the skull. At the mechanistic level, current evidence suggests that ultrasound can influence neuronal excitability predominantly through mechanical interactions with the cell membrane rather than through bulk heating alone. Low-intensity studies have shown transient intracellular Na^{2+} and Ca^{2+} changes together with vesicle exocytosis (Tyler et al., 2008), while more recent work supports a membrane-tension model in which ultrasound deforms the lipid bilayer and engages mechanosensitive ion channels (Y. Zhang, Daday, et al., 2021). In particular, TRPC1 and TRPP2 appear to mediate inward Ca^{2+} flux, which then activates TRPM4, promotes Na^{2+} influx, depolarises the membrane, and can bring neurons towards threshold (S. Yoo et al., 2022a).

Across animal studies, ultrasound has been shown to modulate both cortical and subcortical targets, with reported effects including sensory processing, motor output and seizure-related activity (D'Souza et al., 2020a; Fisher & Gumenchuk, 2018; W. Lee et al., 2018), to name but a few. In these studies, the most commonly used carrier frequencies fall within the sub-MHz range, particularly around 250 – 700 kHz (Kosnoff et al., 2024; P. F. Yang et al., 2021), although higher-frequency protocols are also reported; PD values are often around 0.25 – 5 ms (Brinker et al., 2020; H. Kim et al., 2014), PRF commonly around 100 – 2000 Hz (H. Kim et al., 2015; P. F. Yang et al., 2021), and single SD frequently around 0.05 – 0.4 s (Yuan et al., 2020), with longer patterned paradigms extending to tens or hundreds of seconds.

In humans, tFUS has now been applied to somatosensory, motor, visual, higher-order cortical (Fomenko et al., 2020b; Kosnoff et al., 2024; Legon et al., 2014a; Reznik et al., 2020), and deep targets including the thalamus and hippocampus (Brinker et al., 2020; E. Martin et al., 2025b), with effects reported on sensory-evoked responses, oscillatory activity, motor excitability, perceptual performance, functional connectivity, and in

early clinical work, symptom-related outcomes (Barksdale et al., 2025). Across the human literature reviewed in this thesis, the most commonly used carrier frequencies again cluster around 210 – 700 kHz, with 500 kHz especially frequent in cortical studies; PD are typically around 0.2 – 1 ms, PRF around 300 – 1000 Hz, and sonication durations commonly around 0.2 – 0.5 s in online paradigms, although longer repetitive trains are also used.

Overall, these studies provide compelling evidence that ultrasound can modulate neural systems, but also make clear that the field still operates across a broad and heterogeneous energy range. In the studies reviewed here, reported acoustic intensity (I_{sppa}) span approximately 0.2 - 64.9 W/cm² in animal work, and 0.168 – 35 W/cm² in human studies, largely within the 210 – 700 kHz frequency window. Such variation is likely to contribute to the divergent and sometimes apparently contradictory findings reported across cellular, physiological, and behavioural levels, including facilitatory and suppressive effects under different protocols (Legon, Bansal, et al., 2018; Shamli Oghli et al., 2023; Zeng et al., 2022).

In this sense, the problem is not simply whether ultrasound can modulate neural systems, but rather how to establish comparable acoustic conditions so that consistent outcomes can be obtained across experimental platforms. Without reliable estimation of the delivered acoustic field, interpretation of facilitatory, suppressive or null findings remains limited.

The work presented in this thesis was organised around this problem of acoustic delivery. The first stage established the acoustic source characterisation framework used throughout the later studies. The electromechanical behaviour of the piezoelectric source was linked to measurable acoustic output, this was followed by experimental source characterisation. One important outcome of this work was that source behaviour could not be treated as an ideal geometric abstraction alone; both amplitude and phase required careful characterisation if subsequent transcranial prediction and validation were to remain physically meaningful.

The next stage examined how ex-vivo human skulls modify transmitted ultrasound. We have shown that transmission loss increases with frequency, but also that simple bulk

descriptors such as thickness and SDR are not, by themselves, sufficient to explain intracranial delivery. Local morphology, thickness variation, curvature, insertion angle, and transducer-skull spacing all materially influenced energy loss and focal distortion. This made clear that transcranial delivery is governed not only by attenuation, but also by geometry and interference, and therefore that subject-specific modelling is likely to be beneficial for future in-human studies. However, translation from these ex-vivo measurements to in-human application must also account for factors not fully represented here, including the scalp and other soft tissues, intact cranial geometry, and practical variability in transducer positioning and coupling. To that end, a CT-informed skull analytical model was developed for estimation of optimal transducer placement and transcranial insertion loss (Chapter 5). By retaining the physical transducer and a dynamically segmented three-layer skull representation within a six-layer propagation structure, the model preserved the principal wave-interference behaviour that is often neglected in other analytical approaches. The proposed framework reproduced the main frequency-dependent and oscillatory features of insertion loss across the whole skull surface, while remaining computationally more efficient than full-wave simulation, and should therefore be regarded as a practical and physically interpretable tool for tFUS planning.

From a clinical delivery perspective, once a suitable acoustic window has been identified, numerical simulation becomes important for predicting the resulting intracranial field and the extent of target-engagement variation. The central question in this part of the work was therefore how much uncertainty is introduced by different simulation strategies. Five skull-modelling approaches used across the field were compared against measurement. Although all five approaches reproduced the gross intracranial beam pattern, none achieved uniformly high quantitative accuracy across all metrics. Peak-pressure errors remained moderate, whereas intensity and focal volume errors were substantially larger. Interestingly, the relatively simple linear-mapping model with fixed attenuation often produced lower pressure error than more elaborate modelling approaches, suggesting that additional anatomical detail does not necessarily improve predictive fidelity if the underlying property assignment remains uncertain. This made clear that skull modelling remains one of the dominant sources of uncertainty in tFUS simulation (Chapter 6).

Building on this work, the final stage moved from physical and computational work into a biological setting by examining the feasibility of combining tFUS with hippocampal electrophysiology in a rat model. Using ex vivo human skull specimens, we showed that a 650 kHz cone-coupled system could deliver a focal field at a depth relevant to the hippocampus, although transmission remained sensitive to cone geometry and coupling conditions. In-vivo, pp-DG electrophysiology could be recorded during active sonication, demonstrating that ultrasound delivery and interpretable electrophysiological acquisition could be integrated within the same experimental platform. The fEPSP slope remained stable, while modest changes were observed in PS amplitude and low-frequency spectral power. Because this was designed as a single-animal feasibility study, no population-level inference was attempted.

Seen as a whole, the thesis shows that the practical value of tFUS depends not only on whether ultrasound can influence neural systems, but on whether the delivered field can be characterised and interpreted with sufficient confidence. We approached this problem by understanding piezoceramic vibration and acoustic energy generation, determining acoustic source behaviour experimentally, quantifying skull-induced distortion, developing a rapid analytical predictor, benchmarking full-wave simulation strategies, and testing the system in an in-vivo neuromodulation setting. The thesis therefore contributes a more reliable physical and methodological foundation for future tFUS neuromodulation research. The principal contribution to knowledge is the establishment of an experimentally grounded framework linking source characterisation, skull-mediated transmission, analytical prediction, numerical-model uncertainty, and biological implementation. The work demonstrates that reliable transcranial delivery cannot be inferred from nominal source settings or simplified skull descriptors alone, identifies key sources of uncertainty in acoustic prediction, and provides practical approaches for their characterisation and reduction. In doing so, this thesis advances tFUS towards more measurement-informed, subject-specific, and quantitatively interpretable neuromodulation, providing a stronger physical and methodological foundation for future experimental and clinical studies.

7.2. Future work

One promising next step is to incorporate artificial intelligence into acoustic field prediction. Although analytical and numerical approaches remain indispensable, they also involve an inherent trade-off between physical fidelity and computational cost. Initial work has already been carried out using experimentally measured insertion loss data to learn corrections to simulated transcranial loss (Wang et al., 2026). The next stage would be to move beyond scalar insertion loss prediction and train a model to predict the transmitted acoustic field itself from skull structural input. Ideally, this would be supervised using paired measurements of skull structure and transmitted acoustic fields. However, because acquisition of full experimental field maps is time intensive, a practical intermediate strategy would be to use k-Wave simulated fields as the primary training dataset and then refine or validate the learned model using a smaller set of measured fields. If successful, this approach could provide predictions that are substantially faster while remaining more realistic than full-wave simulation, thereby offering a practical route toward rapid acoustic-field estimation.

A second direction is to translate the present analytical modelling framework and artificial intelligence model into a practical real-time targeting and demonstration system. The analytical model developed in this thesis can already provide a rapid overview of skull transmission performance for a selected RoI, and its computational efficiency makes it suitable for interactive use. A natural extension would be to combine this model with an optical tracking system and a gel head phantom containing thermally responsive markers placed at intended brain target locations. In such a system, a graphical user interface could first present an overview of expected skull transmission performance for a candidate location, and then update the transducer position relative to the target in real time as the transducer is manoeuvred. Once the transducer approaches the skull, the predicted acoustic field could be rapidly registered and superimposed onto a 3D MRI brain image, such that the relative alignment between the focus and the intended target is updated continuously during positioning and verified using the thermally responsive markers. In this way, analytical modelling, artificial intelligence, anatomical imaging, and physical tracking could be integrated into a

practical demonstration of model-guided tFUS targeting. However, AI-based predictions may be unreliable for anatomies or acoustic conditions that differ substantially from the training data. Such predictions should therefore be accompanied by uncertainty assessment and independent physical or numerical validation, rather than being used as the sole basis for safety-critical targeting decisions.

Notwithstanding, several challenges remain before such a system can be implemented robustly. The first is acoustic coupling. In practice, coupling between the transducer and the skull is not perfectly stable, and small changes in gel layer thickness can alter the transmitted field. The second is focal geometry. Even when target alignment is satisfactory in a geometric sense, the focal region produced by a single-element transducer may remain axially elongated relative to the intended neural target, so that acceptable spatial registration may still correspond to excessive acoustic coverage beyond the target volume. In this respect, a real-time demonstration system would not only illustrate the potential of model-guided placement, but would also expose the present limitations of current transducer configurations.

A logical next step is therefore to investigate source configurations that reduce focal elongation and improve confinement at depth. One promising approach is a dual-transducer arrangement in which two focused beams are placed approximately orthogonal to one another (S. Kim et al., 2021), so that their intersection defines a smaller effective focal region than either transducer produces alone. If such a system can be implemented with adequate registration and coupling control, it may offer a practical means of reducing exposure to surrounding tissue while preserving sufficient energy at the intended target. However, a dual-transducer system would introduce additional challenges, including maintaining accurate spatial registration between the two foci and accounting for different skull transmission paths and phase aberrations. These factors could reduce the intended overlap of the two focal regions and would require careful calibration and subject-specific planning.

A further priority is to extend the biological work into a more rigorous and controlled experimental framework. The work made clear that stronger causal interpretation will require sham sonication and larger sample sizes. These refinements would make it possible to determine whether the descriptive effects observed here reflect genuine

modulation of hippocampal synaptic and network function. More broadly, the future development of tFUS is likely to depend on bringing together three strands that are still too often treated separately: realistic and efficient field prediction, practical targeting and registration, and controlled biological validation. The work in this thesis provides a basis for each of these. The next stage is to integrate them into a framework that is not only physically informed, but also sufficiently fast, practical, and reliable for routine neuromodulation research.

7.3. Conclusion

This thesis set out to address a central problem in tFUS neuromodulation: how to move from the conceptual promise of the technique to a framework in which acoustic delivery is predicted and validated with sufficient confidence for meaningful experimental interpretation. The work has shown that this problem is not trivial, skull transmission depends on more than mean attenuation, simulation accuracy depends on more than geometric detail, and apparently simple factors such as transducer-skull spacing can introduce substantial uncertainty into the delivered field.

At the same time, the thesis has shown that these limitations can be approached systematically. Skull-induced loss and distortion can be quantified and related to structural features, analytical models can capture the dominant interference behaviour with useful accuracy and high efficiency, and full-wave simulations can reproduce major aspects of intracranial field structure while also revealing the uncertainty introduced by different skull modelling strategies. Finally, a tFUS system can be integrated with in-vivo hippocampal electrophysiology in a way that preserves interpretable recordings.

The main conclusion is therefore not simply that tFUS is promising, but that its promise becomes significantly more useful when the physical problem of transcranial delivery is treated as central rather than secondary. If tFUS is to mature as a neuromodulation tool, its biological effects should be interpreted alongside the acoustic uncertainties that

shape them. By linking transcranial measurement, analytical prediction, simulation benchmarking, and in-vivo feasibility within a single body of work, this thesis contributes towards that goal and provides a more reliable foundation for future tFUS neuromodulation research.

APPENDIX A

Appendix Table-1 Representative focused ultrasound neuromodulation studies and acoustic exposure parameters. NR = not reported in the reviewed section. 'calc.' indicates duty cycle calculated from $PD \times PRF$ where sufficient information was available.

Target	Species	f	Pressure	Intensity	PD	PRF	DC	SD/ISI	Compact reported outcome	Reference
Amygdala	Human mood, anxiety and trauma symptoms	650 kHz	NR	Isppa 14.4 W/cm ²	5 ms	10 Hz	5%	30 s	Reduced amygdala activation and connected hippocampal activity.	(Barksdale et al., 2025)
SCC	Human TRD	500 kHz	NR	NR	4.5 ms	14 Hz	5%	5 s	Altered prefrontal and hippocampal connectivity.	(Attali et al., 2025)
Ion-channel mechanism	Cellular / neuronal preparation	300 kHz	NR	approx. 15 W/cm ²	NR	Pulsed	NR	500 ms	Ca ²⁺ influx via TRPC1/TRPP2; TRPM4-mediated Na ⁺ influx and depolarisation.	(S. Yoo et al., 2022a)
S1	Mouse	510 kHz	170 kPa	Isppa 0.69 W/cm ²	0.5 ms	1 kHz	NR; 50% calc.	1 s	Earlier sensory-evoked responses; altered Ca ²⁺ activity patterns.	(Fisher & Gumenchuk, 2018)
S1	Mouse	2 MHz	1.2 MPa	Isppa 46 W/cm ²	0.3 ms	1 kHz	NR; 30% calc.	6 s	Local spiking changes and lateralised head-turning responses.	(G. Li et al., 2019)
S1	Macaque	250 kHz	247 kPa	Isppa 2 W/cm ²	0.25 ms	2 kHz	NR; 50% calc.	SD 0.3 s; ISI 0.7 s	Suppressed tactile-evoked BOLD at target and modulated downstream regions.	(P. F. Yang et al., 2021)
S1	Human	500 kHz	800 kPa	Isppa 23.87 W/cm ²	0.36 ms	1 kHz	NR; 36% calc.	0.5 s	Attenuated SEPs, altered oscillatory power, improved tactile discrimination.	(Legon et al., 2014)
S1	Human	500 kHz	800 kPa	Isppa 23.87 W/cm ²	0.36 ms	1 kHz	NR; 36% calc.	0.5 s	Altered intrinsic and evoked phase dynamics, especially beta-band activity.	(Mueller et al., 2014)

APPENDIX A

Target	Species	f	Pressure	Intensity	PD	PRF	DC	SD/ISI	Compact reported outcome	Reference
S1 and S2	Human	210 kHz	NR	Isppa 35 W/cm ²	1 ms	500 Hz	NR; 50% calc.	0.5 s	Transient contralateral tactile percept during dual-site stimulation.	(W. Lee, Kim, et al., 2016)
S1	Human	500 kHz	286 kPa	Isppa 1.1 W/cm ²	0.2 ms	300 Hz	NR; 6% calc.	0.5 s	Improved vibration discrimination.	(C. Liu et al., 2021)
Motor cortex	Rat	350 kHz	NR	Isppa 4.9-5.6 W/cm ²	1-5 ms	100-500 Hz	NR	0.3 s	Motor responses.	(H. Kim et al., 2014)
Motor cortex	Rat	600 kHz	NR	Isppa 14.9 W/cm ²	1 ms	500 Hz	NR; 50% calc.	0.3 s	Movements across body regions.	(W. Lee et al., 2018)
M1 / thalamic projection region	Sheep	250 kHz	NR	Isppa 5.2 W/cm ²	0.5-2 ms	approx. 350-1400 Hz	approx. 70% calc.	0.2 s	Selective contralateral hindlimb EMG.	(H. C. Kim et al., 2022)
M1	Human	500 kHz	NR	Isppa 17 W/cm ²	NR	1 kHz	NR	0.5 s	Reduced MEP amplitude and intracortical facilitation.	(Legon, Bansal, et al., 2018)
M1	Human	500 kHz	NR	Isppa 30 W/cm ²	30 ms	10 Hz	NR; 30% calc.	40 s	Sustained MEP suppression.	(Nakajima et al., 2022)
M1	Human	500 kHz	NR	NR	20 ms	5 Hz	NR; 10% calc.	80 s	increased MEP amplitude; reduced intracortical inhibition.	(Zeng et al., 2022)
M1	Human	500 kHz	NR	NR	20 ms	5 Hz	NR; 10% calc.	80 s	Sustained MEP increase; altered alpha/beta activity and motor-network connectivity.	(Sarica et al., 2022)
M1	Human	500 kHz	NR	Isppa approx. 8 W/cm ²	NR	100 Hz	NR	SD 0.5 s; ISI 8 s; 15 min	Increased ipsilateral excitability; transiently shifted interhemispheric balance.	(Y. Zhang, Ren, et al., 2021)
M1	Human	800 kHz	NR	Isppa approx. 1.2 W/cm ²	NR	NR	NR	SD 2 s; ISI 2 s	Improved hand tapping, shorter MEP latency and higher MEP amplitude.	(M. F. Zhang et al., 2022)
M1	Human	500 kHz	NR	Isppa 3.9 W/cm ²	NR	1 kHz	NR	0.3-0.5 s	No concurrent EMG modulation or excitability change.	(Heimbuch et al., 2022)

APPENDIX A

Target	Species	f	Pressure	Intensity	PD	PRF	DC	SD/ISI	Compact reported outcome	Reference
V1	Rat	690 kHz	NR	Isppa 3 W/cm ²	0.5 ms	100 Hz	NR; 5% calc.	150 s	Transient suppression of light-evoked responses.	(H. Kim et al., 2015)
FEF	Macaque	320 kHz	350 kPa	Isppa 4 W/cm ²	100 ms	0.1 Hz	NR; 1% calc.	NR	Behavioural slowing during antisaccade task.	(Deffieux et al., 2013)
FEF to V4	Macaque	700 kHz	NR	Isppa 10.4 W/cm ²	5 ms	100 Hz	NR; 50% calc.	0.3 s	Reduced V4 spiking.	(Yu et al., 2024)
V1	Human	270 kHz	NR	Isppa avg. 3 W/cm ²	1 ms	500 Hz	NR; 50% calc.	0.3 s	Stimulus-locked EEG responses and diffuse percept.	(W. Lee, Kim, et al., 2016)
V5	Human	700 kHz	200 kPa	Isppa 0.168 W/cm ²	0.2 ms	3 kHz	NR; 60% calc.	0.5 s	Improved online spelling performance in visual-evoked-potential BCI paradigm.	(Kosnoff et al., 2024)
Auditory cortex	Rat / swine	230 kHz	39.7 kPa	Isppa 2.3 W/cm ²	100 ms	0.33 Hz	NR; 3.3% calc.	52 s	Suppressed AEP amplitudes in rats; comparable suppression in swine.	(Daniels et al., 2018)
PFC	Human	500 kHz	approx. 306-600 kPa	Isppa approx. 3.15-16.2 W/cm ²	0.065-0.125 ms	40 Hz	approx. 0.26-0.5% calc.	30-120 s	Improved global affect, reduced anxiety and altered resting-state connectivity.	(Reznik et al., 2020)
Thalamus	Rat	650 kHz	NR	Isppa 6 W/cm ²	0.5 ms	100 Hz	NR; 5% calc.	NR	Shortened emergence from anaesthesia.	(S. S. Yoo et al., 2011)
Thalamus	Rat	690 kHz	270 kPa	Isppa 2.6 W/cm ²	0.5 ms	100 Hz	NR; 5% calc.	NR	Reduced epileptiform bursts and seizure severity.	(Min et al., 2011)
Thalamus	Rat	500 kHz	pmax 530 kPa	NR	0.8-3 ms	100 Hz	NR; 8-30% calc.	100-600 s	Seizure suppression.	(S. G. Chen et al., 2020)
Thalamus	Swine	1.14 MHz	NR	Isppa 25-30 W/cm ²	43.7 ms	10 Hz	NR; 43.7% calc.	40 s	Reversibly suppressed SSEPs.	(Dallapiazza et al., 2017)
Thalamus	Human disorders of consciousness	650 kHz	NR	Isppa 14.39 W/cm ²	0.5 ms	100 Hz	NR; 5% calc.	30 s	Behavioural improvement.	(Cain et al., 2021)

APPENDIX A

Target	Species	f	Pressure	Intensity	PD	PRF	DC	SD/ISI	Compact reported outcome	Reference
Thalamus	Human	650 kHz	NR	Isppa 14.38 W/cm ²	5 ms	10 Hz	NR; 5% calc.	30 s	Reduced thermal pain sensitivity.	(Badran et al., 2020)
S1	Human	250 kHz	NR	Isppa 3.4-4.1 W/cm ²	0.5-2 ms	NR	70%	0.2 s	EEG responses; increased thalamic-network connectivity.	(H. C. Kim et al., 2023)
LGN	Human	555 kHz	775 kPa	NR	300 ms every 3 s	approx. 0.33 Hz	approx. 10% calc.	NR	Increased downstream V1 fMRI activity.	(E. Martin et al., 2025b)
Hippocampus	Mice	500 kHz	approx. 230 kPa	Isppa 1.75 W/cm ²	NR	1 kHz	40%	0.4 s	Increased hippocampal theta power.	(H. Yang et al., 2020)
Hippocampus	Human	548 kHz	NR	Isppa 1-6.25 W/cm ²	0.72-1 ms	500 Hz	36-50%	SD 0.5 s; ISI 7 s	Safe delivery across multiple sessions.	(Brinker et al., 2020)
Hippocampus	Human	250 kHz	NR	Isppa 0.6-6.54 W/cm ²	20 ms	2 Hz	4%	0.4 s	No detectable BBB opening; increased glucose metabolism and modest memory improvement.	(Jeong et al., 2022)
Amygdala and ACC	Macaque	250 kHz	NR	Isppa 64.9 and 18.8 W/cm ²	30 ms	NR	NR	NR	Reversible connectivity changes.	(Folloni et al., 2019)
Amygdala/Entorhinal cortex	Human	650 kHz	NR	NR	0.5-5 ms	10-100 Hz	5%	NR	Selective perfusion increase and altered target-region connectivity.	(Kuhn et al., 2023)

APPENDIX B

Perturbation derivation of the cycle-averaged conservative momentum

Starting from the conservative momentum equation,

$$\frac{\partial(\rho \mathbf{v})}{\partial t} + \nabla \cdot (p \mathbf{I} + \rho \mathbf{v} \mathbf{v}) = 0, \quad (\text{A1})$$

the cycle-averaged second-order momentum-flux tensor may be obtained using the perturbation expansion

$$\begin{aligned} p &= p_0 + \varepsilon p_1 + \varepsilon^2 p_2 + \dots, \\ \rho &= \rho_0 + \varepsilon \rho_1 + \varepsilon^2 \rho_2 + \dots, \\ \mathbf{v} &= \varepsilon \mathbf{v}_1 + \varepsilon^2 \mathbf{v}_2 + \dots \end{aligned} \quad (\text{A2})$$

with p_0 and ρ_0 uniform equilibrium pressure and density, respectively, and $\mathbf{v}_0 = 0$. terms of order ε^3 and higher are neglected.

Substituting Eq. (A2) into Euler's equation,

$$\rho \left(\frac{\partial \mathbf{v}}{\partial t} + (\mathbf{v} \cdot \nabla) \mathbf{v} \right) + \nabla p = 0, \quad (\text{A3})$$

$$\begin{aligned} \rho \left(\frac{\partial \mathbf{v}}{\partial t} + (\mathbf{v} \cdot \nabla) \mathbf{v} \right) &= (\rho_0 + \varepsilon \rho_1 + \varepsilon^2 \rho_2) \left[\varepsilon \frac{\partial \mathbf{v}_1}{\partial t} + \varepsilon^2 \frac{\partial \mathbf{v}_2}{\partial t} + \varepsilon^2 (\mathbf{v}_1 \cdot \nabla) \mathbf{v}_1 \right] \\ &= \varepsilon \rho_0 \frac{\partial \mathbf{v}_1}{\partial t} + \varepsilon^2 \left[\rho_0 \frac{\partial \mathbf{v}_2}{\partial t} + \rho_1 \frac{\partial \mathbf{v}_1}{\partial t} + \rho_0 (\mathbf{v}_1 \cdot \nabla) \mathbf{v}_1 \right]; \end{aligned}$$

$$\begin{aligned} \nabla p &= \nabla(p_0 + \varepsilon p_1 + \varepsilon^2 p_2) \\ &= \varepsilon \nabla p_1 + \varepsilon^2 \nabla p_2, \end{aligned}$$

and collecting first-order terms gives

$$\rho_0 \frac{\partial \mathbf{v}_1}{\partial t} + \nabla p_1 = 0. \quad (\text{A4})$$

Collecting second-order terms gives

$$\rho_0 \frac{\partial \mathbf{v}_2}{\partial t} + \rho_1 \frac{\partial \mathbf{v}_1}{\partial t} + \rho_0 (\mathbf{v}_1 \cdot \nabla) \mathbf{v}_1 + \nabla p_2 = 0. \quad (\text{A5})$$

To simplify the nonlinear convective term, the standard vector identity

$$(\mathbf{v}_1 \cdot \nabla) \mathbf{v}_1 = \nabla \frac{v_1^2}{2} - \mathbf{v}_1 \times (\nabla \times \mathbf{v}_1) \quad (\text{A6})$$

is used. For an irrotational first-order acoustic field,

$$\nabla \times \mathbf{v}_1 = 0, \quad (\text{A7})$$

and therefore

$$(\mathbf{v}_1 \cdot \nabla) \mathbf{v}_1 = \nabla \frac{v_1^2}{2}. \quad (\text{A8})$$

Using the first-order isentropic relation

$$p_1 = c_0^2 \rho_1, \quad (\text{A9})$$

together with Eq. (A4), substituting Eqs. (A8) and (A9) into Eq. (A5) yields

$$\begin{aligned} \nabla p_2 &= \left(-\frac{p_1}{c_0^2} \cdot \left(-\nabla \frac{p_1}{\rho_0} \right) \right) - \rho_0 \nabla \frac{v_1^2}{2} - \rho_0 \frac{\partial \mathbf{v}_2}{\partial t} \\ &= \nabla \left(\frac{p_1^2}{2\rho_0 c_0^2} - \frac{\rho_0 v_1^2}{2} \right) - \rho_0 \frac{\partial \mathbf{v}_2}{\partial t}. \end{aligned} \quad (\text{A10})$$

For periodic steady state, the cycle average of the second-order time derivative vanishes, the second-order scalar term therefore becomes

$$\langle p_2 \rangle = \left\langle \frac{p_1^2}{2\rho_0 c_0^2} - \frac{\rho_0 v_1^2}{2} \right\rangle, \quad (\text{A11})$$

Next, expanding the advective momentum flux gives

$$\rho \mathbf{v} \mathbf{v} = (\rho_0 + \varepsilon \rho_1 + \varepsilon^2 \rho_2) (\varepsilon \mathbf{v}_1 + \varepsilon^2 \mathbf{v}_2) (\varepsilon \mathbf{v}_1 + \varepsilon^2 \mathbf{v}_2), \quad (\text{A12})$$

so that the cycle average of second-order contribution is

$$\langle \rho \mathbf{v} \mathbf{v} \rangle^{(2)} = \rho_0 \langle \mathbf{v}_1 \mathbf{v}_1 \rangle. \quad (\text{A13})$$

The cycle-averaged second-order momentum-flux tensor therefore becomes

$$\begin{aligned}\langle \mathbf{T}^{(2)} \rangle &= \langle p_2 \rangle \mathbf{I} + \langle \rho \mathbf{v} \mathbf{v} \rangle^{(2)} \\ &= \left\langle \frac{p_1^2}{2\rho_0 c_0^2} - \frac{\rho_0 v_1^2}{2} \right\rangle \mathbf{I} + \rho_0 \langle \mathbf{v}_1 \mathbf{v}_1 \rangle.\end{aligned}\tag{A14}$$

For a monochromatic plane progressive wave, $p_1 = \rho_0 c_0 v_1$, so the cycle-averaged isotropic second-order term vanishes,

$$\left\langle \frac{p_1^2}{2\rho_0 c_0^2} - \frac{\rho_0 v_1^2}{2} \right\rangle = 0.\tag{A15}$$

Hence,

$$\langle \mathbf{T}^{(2)} \rangle = \rho_0 \langle \mathbf{v}_1 \mathbf{v}_1 \rangle.\tag{A16}$$

This result yields the simplified radiation-force relation reported in Eq. (3.2.13) of the main text.

APPENDIX C

Plane-wave relation between first-order pressure, particle velocity and intensity

Starting from the first-order momentum equation,

$$\rho_0 \frac{\partial \mathbf{v}_1}{\partial t} + \nabla p_1 = 0, \quad (\text{B1})$$

assume a monochromatic plane progressive wave of the form

$$p_1(\mathbf{r}, t) = \hat{p} e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)}, \mathbf{v}_1(\mathbf{r}, t) = \hat{\mathbf{v}} e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)}, \quad (\text{B2})$$

where $\mathbf{r}$ is the position vector and $|\mathbf{k}| = \omega/c_0$. Taking the temporal derivative at fixed position and the spatial gradient at fixed time gives

$$\begin{aligned} \frac{\partial \mathbf{v}_1}{\partial t} &= \hat{\mathbf{v}} \frac{\partial}{\partial t} e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)} = \hat{\mathbf{v}} (-i\omega) e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)} \\ &= -i\omega \hat{\mathbf{v}}, \end{aligned} \quad (\text{B3})$$

$$\begin{aligned} \nabla p_1 &= \hat{p} \nabla e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)} \\ &= \hat{p} e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)} \nabla i(\mathbf{k} \cdot \mathbf{r} - \omega t) \\ &= \hat{p} e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)} \nabla i(\mathbf{k} \cdot \mathbf{r}) \\ &= \hat{p} e^{i(\mathbf{k} \cdot \mathbf{r} - \omega t)} i \left(\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z} \right) (k_x \cdot x + k_y \cdot y + k_z \cdot z) \\ &= i\mathbf{k} \hat{p} \end{aligned} \quad (\text{B4})$$

Substituting Eqs. (B3) and (B4) into Eq. (B1) gives the complex-amplitude relation

$$\rho_0 (-i\omega \hat{\mathbf{v}}) + i\mathbf{k} \hat{p} = 0 \quad (\text{B5})$$

Taking the longitudinal scalar component along the propagation direction gives

$$\hat{v} = \frac{\hat{p}}{\rho_0 c_0}. \quad (\text{B6})$$

Therefore, in physical variables,

$$p_1 = \rho_0 v_1 c_0. \quad (\text{B7})$$

The instantaneous acoustic intensity is

$$i(t) = p_1(t)v_1(t), \quad (\text{B8})$$

and taking the time average of the instantaneous product over one acoustic cycle gives the time-averaged intensity

$$I = \langle p_1 v_1 \rangle = \frac{1}{2} \hat{p} \hat{v}. \quad (\text{B9})$$

Using Eq. (B6), this becomes

$$I = \frac{\hat{p}^2}{2\rho_0 c_0} = \frac{p_{1,rms}^2}{\rho_0 c_0}. \quad (\text{B10})$$

Integration over the beam cross-section gives the total acoustic power,

$$P = \iint_A I dA, \quad (\text{B11})$$

APPENDIX D

Skull physical property maps for S1 to S20

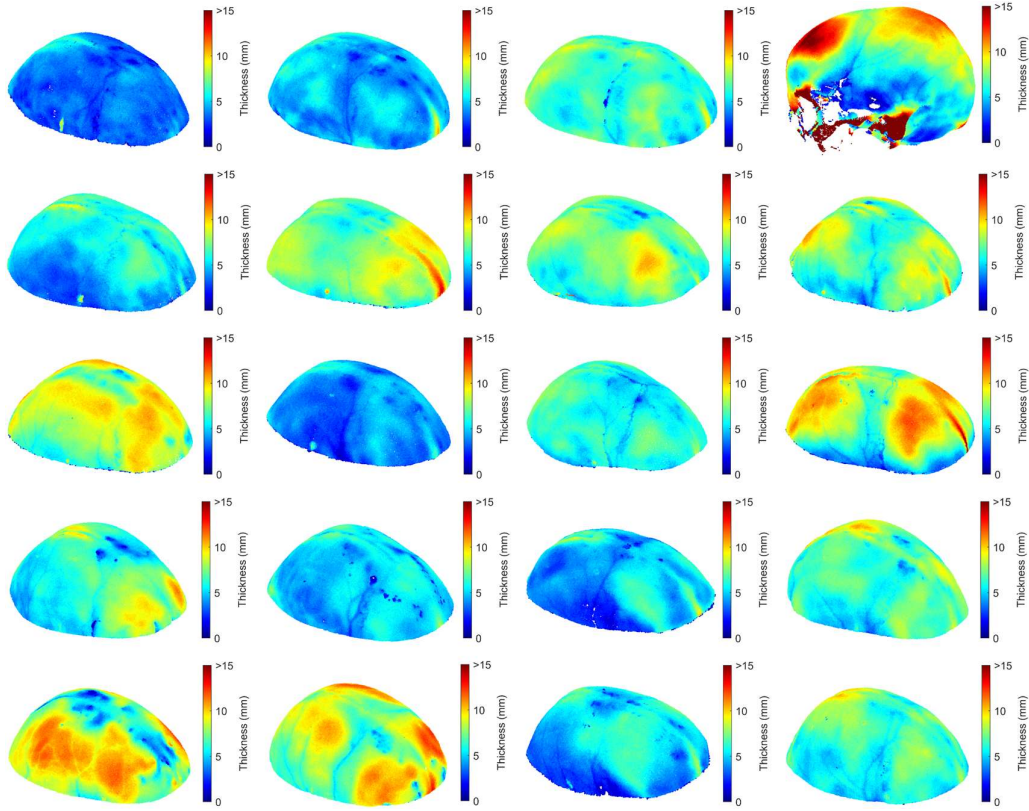


Figure D-1 Skull thickness map of S1 to S20 in the same order of insertion loss plots in the paper.

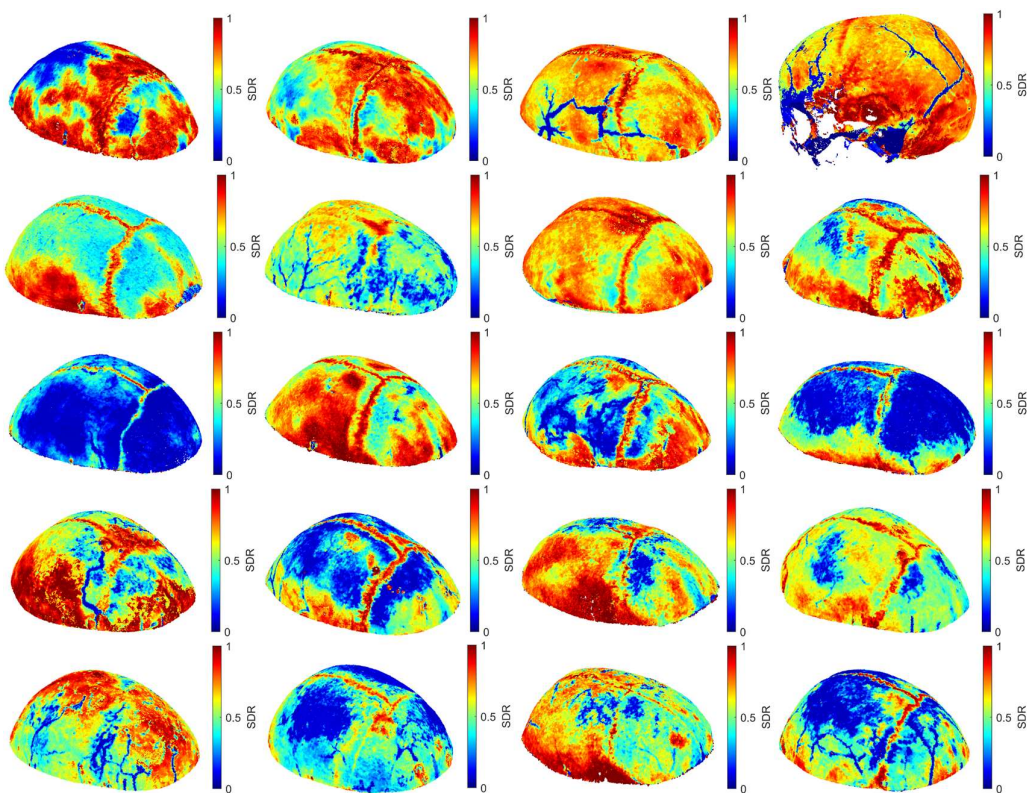


Figure D-2 Skull SDR map of S1 to S20.

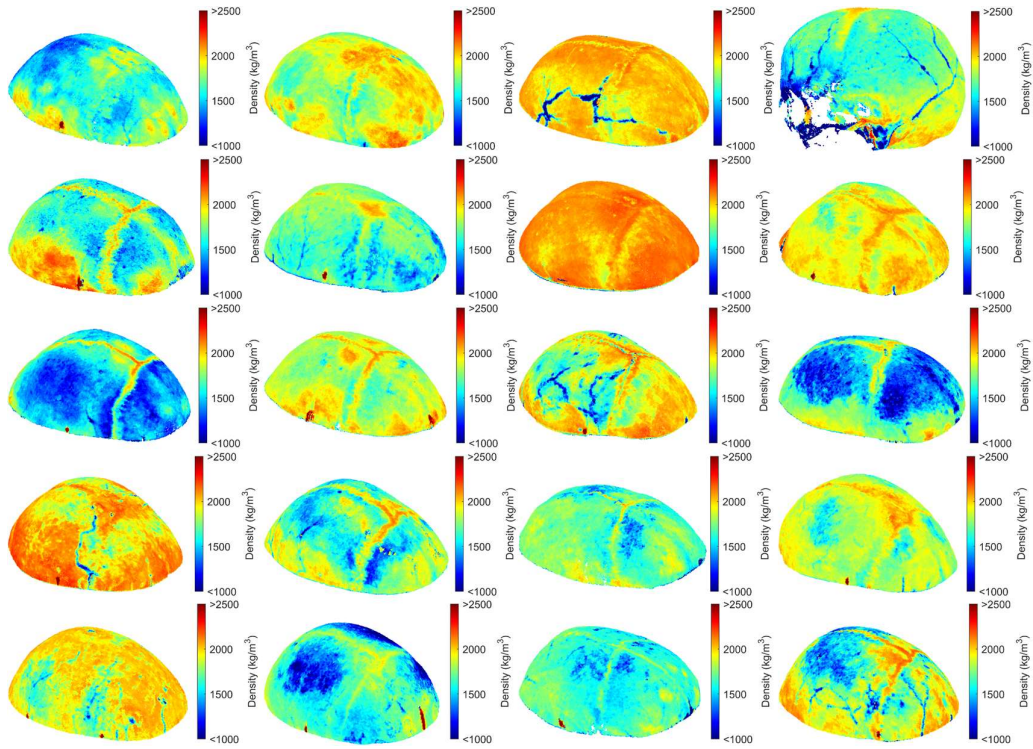


Figure D -1 Skull density map of S1 to S20

APPENDIX E

Transmission and reflection coefficient

At skull-water interface:

$$V_{ij} = \frac{Z_j + Z_s \sin^2 2\theta_{is} - Z_i \sin^2 2\theta_{is}}{Z_j + Z_s \sin^2 2\theta_{is} + Z_i \sin^2 2\theta_{is}}$$

$$W_{ij} = \frac{(1 - V_{ij}) \tan \theta_j \cot \theta_i}{\cos 2\theta_{is}},$$

where

$$Z_i = \frac{\rho_i c_i}{\cos \theta_i}, \quad Z_s = \frac{\rho_i c_{is}}{\cos \theta_{is}}, \quad Z_j = \frac{\rho_j c_j}{\cos \theta_j}, \quad i = 2, j = 1.$$

At skull layers interface:

$$V_{ij} = [(b\xi_i - d\xi_j)F - (a + e\xi_i\eta_j)H\zeta^2]D^{-1}$$

$$W_{ij} = \left(\frac{Z_j}{Z_i}\right) 2\rho_i \xi_i \left(\frac{c_i}{c_j}\right) F D^{-1},$$

where

$$a = \gamma_j - \gamma_i, \quad b = \gamma_j + \chi_i \zeta, \quad d = \gamma_j + \chi_i \zeta, \quad e = 2\rho_j c_{js}^2 - \rho_i c_{is}^2,$$

$$\chi_l = 2\rho_l c_l \zeta, \quad \gamma_l = \rho_l (1 - 2c_l^2 \zeta^2), \quad \xi_l = \frac{\cos \theta_l}{c_l}, \quad \eta_l = \frac{\cos \theta_{ls}}{c_{ls}},$$

$$l = i, j = 2, 3, 4$$

$$E = b\xi_i + d\xi_j, \quad F = b\eta_i + d\eta_j, \quad G = a - e\xi_i\eta_j,$$

$$H = a - e\xi_i\eta_j, \quad D = EF + GH\zeta^2.$$

APPENDIX F

Measured, simulated and analysed pressure insertion loss levels of 20 skulls' RoI
(normalised to 1)

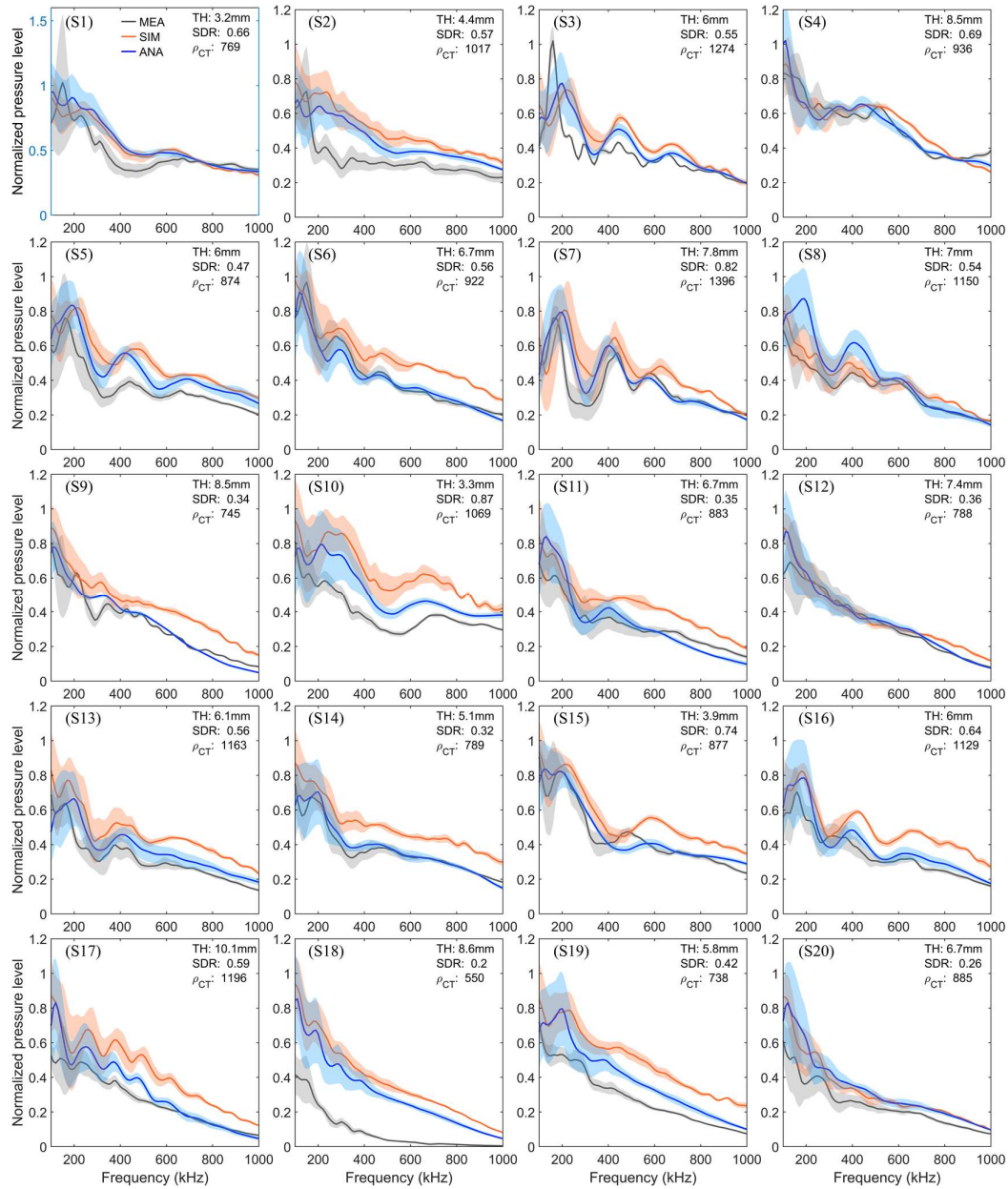


Figure F-1 Measured, simulated and analysed pressure insertion loss levels of 20 skulls' RoI, normalised to 1.

Bibliography

- Aarnio, J., Clement, G. T., & Hynynen, K. (2005). A new ultrasound method for determining the acoustic phase shifts caused by the skull bone. *Ultrasound in Medicine & Biology*, 31(6), 771–780. <https://doi.org/10.1016/J.ULTRASMEDBIO.2005.01.019>
- Abbott, C. C., Miller, J., Farrar, D., Argyelan, M., Lloyd, M., Squillaci, T., Kimbrell, B., Ryman, S., Jones, T. R., Upston, J., Quinn, D. K., Peterchev, A. V., Erhardt, E., Datta, A., McClintock, S. M., & Deng, Z. De. (2024). Amplitude-determined seizure-threshold, electric field modeling, and electroconvulsive therapy antidepressant and cognitive outcomes. *Neuropsychopharmacology*, 49(4), 640–648. <https://doi.org/10.1038/S41386-023-01780-4>;TECHMETA=57,59;SUBJMETA=1414,308,476,575,692,699;KWRD=DEPRESSION,TRANSLATIONAL+RESEARCH
- Abraham, W. C., Jones, O. D., & Glanzman, D. L. (2019). Is plasticity of synapses the mechanism of long-term memory storage? *Npj Science of Learning* 2019 4:1, 4(1), 9-. <https://doi.org/10.1038/s41539-019-0048-y>
- Adams, C., Jones, R. M., Yang, S. D., Kan, W. M., Leung, K., Zhou, Y., Lee, K. U., Huang, Y., & Hynynen, K. (2021). Implementation of a Skull-Conformal Phased Array for Transcranial Focused Ultrasound Therapy. *IEEE Transactions on Biomedical Engineering*, 68(11), 3457–3468. <https://doi.org/10.1109/TBME.2021.3077802>
- Ai, L., Bansal, P., Mueller, J. K., & Legon, W. (2018). Effects of transcranial focused ultrasound on human primary motor cortex using 7T fMRI: a pilot study. *BMC Neuroscience* 2018 19:1, 19(1), 56-. <https://doi.org/10.1186/s12868-018-0456-6>
- Aksić, M., Radonjić, N. V., Aleksić, D., Jevtić, G., Marković, B., Petronijević, N., Radonjić, V., & Filipović, B. (2013). Long-term effects of the maternal deprivation on the volume and number of neurons in the rat neocortex and hippocampus. *Acta Neurobiologiae Experimentalis*, 73(3), 394–403. <https://doi.org/10.55782/ANE-2013-1946>
- Aksoy-Aksel, A., & Manahan-Vaughan, D. (2013). The temporoammonic input to the hippocampal CA1 region displays distinctly different synaptic plasticity compared to the Schaffer collateral input in vivo: Significance for synaptic information processing. *Frontiers in Synaptic Neuroscience*, 5(AUG). <https://doi.org/10.3389/fnsyn.2013.00005>
- Ammi, A. Y., Mast, T. D., Huang, I. H., Abruzzo, T. A., Coussios, C. C., Shaw, G. J., & Holland, C. K. (2008). Characterization of Ultrasound Propagation Through Ex-vivo Human Temporal Bone. *Ultrasound in Medicine and Biology*, 34(10), 1578–1589. <https://doi.org/10.1016/j.ultrasmedbio.2008.02.012>
- Andrew Drainville, R., Chatillon, S., Moore, D., Snell, J., Padilla, F., Lafon, C., & Express Lett, J. (2023). A simulation study on the sensitivity of transcranial ray-

- tracing ultrasound modeling to skull properties. *The Journal of the Acoustical Society of America*, 154(2), 1211–1225. <https://doi.org/10.1121/10.0020761>
- Angla, C., Chouh, H., Mondou, P., Toullelan, G., Perlin, K., Brulon, V., De Schlichting, E., Larrat, B., Gennisson, J. L., & Chatillon, S. (2024). New semi-analytical method for fast transcranial ultrasonic field simulation. *Physics in Medicine & Biology*, 69(9), 095017. <https://doi.org/10.1088/1361-6560/AD3882>
- Arns, M., & Fitzgerald, P. B. (2024). Somatic Treatments and Neuromodulation in Psychiatry. *Tasman's Psychiatry, Fifth Edition*, 4493–4536. https://doi.org/10.1007/978-3-030-51366-5_144
- Arulpragasam, A. R., Theyel, B., Barredo, J., Pouille, F., Gillotti, J. G., Greenberg, B. D., Wout-Frank, M. van 't, & Philip, N. S. (2026). Low Intensity Focused Ultrasound Neuromodulation in Psychiatric Disorders: Mechanisms, Models, and Missing Links. *Biological Psychiatry*. <https://doi.org/10.1016/J.BIOPSYCH.2026.02.019>
- Attali, D., Tiennot, T., Manuel, T. J., Daniel, M., Houdouin, A., Annic, P., Dizeux, A., Haroche, A., Dadi, G., Henensal, A., Moyal, M., Le Berre, A., Paolillo, C., Charron, S., Debacker, C., Lui, M., Lekcir, S., Mancusi, R., Gallarda, T., ... Plaze, M. (2025). Deep transcranial ultrasound stimulation using personalized acoustic metamaterials improves treatment-resistant depression in humans. *Brain Stimulation*, 18(3), 1004–1014. <https://doi.org/10.1016/J.BRS.2025.04.018>
- Attali, D., Tiennot, T., Schafer, M., Fouragnan, E., Sallet, J., Caskey, C. F., Chen, R., Darmani, G., Bubrick, E. J., Butler, C., Stagg, C. J., Klein-Flügge, M., Verhagen, L., Yoo, S. S., Pauly, K. B., & Aubry, J. F. (2023). Three-layer model with absorption for conservative estimation of the maximum acoustic transmission coefficient through the human skull for transcranial ultrasound stimulation. *Brain Stimulation*, 16(1), 48–55. <https://doi.org/10.1016/j.brs.2022.12.005>
- Aubry, J.-F., Bates, O., Boehm, C., Butts Pauly, K., Christensen, D., Cueto, C., Gélât, P., Guasch, L., Jaros, J., Jing, Y., Jones, R., Li, N., Marty, P., Montanaro, H., Neufeld, E., Pichardo, S., Pinton, G., Pulkkinen, A., Stanziola, A., ... elat, P. G. (2022). Benchmark problems for transcranial ultrasound simulation: Intercomparison of compressional wave models. *The Journal of the Acoustical Society of America*, 152(2), 1003–1019. <https://doi.org/10.1121/10.0013426>
- Aubry, J.-F., Tanter, ; M, Pernot, ; M, Thomas, J.-L., Fink, ; M, Tanter,) M, Pernot, M., & Fink, M. (2003). Experimental demonstration of noninvasive transskull adaptive focusing based on prior computed tomography scans. *The Journal of the Acoustical Society of America*, 113(1), 84–93. <https://doi.org/10.1121/1.1529663>
- Bachtold, M. R., Rinaldi, P. C., Jones, J. P., Reines, F., & Price, L. R. (1998). Focused Ultrasound Modifications of Neural Circuit Activity in a Mammalian Brain. *Ultrasound in Medicine & Biology*, 24(4), 557–565. [https://doi.org/10.1016/S0301-5629\(98\)00014-3](https://doi.org/10.1016/S0301-5629(98)00014-3)
- Badran, B. W., Caulfield, K. A., Stomberg-Firestein, S., Summers, P. M., Dowdle, L. T., Savoca, M., Li, X., Austelle, C. W., Short, E. B., Borckardt, J. J., Spivak, N.,

- Bystritsky, A., & George, M. S. (2020). Sonication of the anterior thalamus with MRI-Guided transcranial focused ultrasound (tFUS) alters pain thresholds in healthy adults: A double-blind, sham-controlled study. *Brain Stimulation*, 13(6), 1805–1812. <https://doi.org/10.1016/j.brs.2020.10.007>
- Bakermans, J. J. W., Warren, J., Whittington, J. C. R., & Behrens, T. E. J. (2025). Constructing future behavior in the hippocampal formation through composition and replay. *Nature Neuroscience* 2025 28:5, 28(5), 1061–1072. <https://doi.org/10.1038/s41593-025-01908-3>
- Bancel, T., Houdouin, A., Annic, P., Rachmilevitch, I., Shapira, Y., Tanter, M., & Aubry, J. F. (2021a). Comparison between Ray-Tracing and Full-Wave Simulation for Transcranial Ultrasound Focusing on a Clinical System Using the Transfer Matrix Formalism. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, 68(7), 2554–2565. <https://doi.org/10.1109/TUFFC.2021.3063055>,
- Bancel, T., Houdouin, A., Annic, P., Rachmilevitch, I., Shapira, Y., Tanter, M., & Aubry, J. F. (2021b). Comparison between Ray-Tracing and Full-Wave Simulation for Transcranial Ultrasound Focusing on a Clinical System Using the Transfer Matrix Formalism. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, 68(7), 2554–2565. <https://doi.org/10.1109/TUFFC.2021.3063055>
- Bari, B. A., Chokshi, V., & Schmidt, K. (2019). Locus coeruleus-norepinephrine: basic functions and insights into Parkinson’s disease. *Neural Regeneration Research*, 15(6), 1006. <https://doi.org/10.4103/1673-5374.270297>
- Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). NON-INVASIVE MAGNETIC STIMULATION OF HUMAN MOTOR CORTEX. *The Lancet*, 325(8437), 1106–1107. [https://doi.org/10.1016/S0140-6736\(85\)92413-4](https://doi.org/10.1016/S0140-6736(85)92413-4)
- Barksdale, B. R., Enten, L., DeMarco, A., Kline, R., Doss, M. K., Nemeroff, C. B., & Fonzo, G. A. (2025). Low-intensity transcranial focused ultrasound amygdala neuromodulation: a double-blind sham-controlled target engagement study and unblinded single-arm clinical trial. *Molecular Psychiatry*, 30(10), 4497–4511. <https://doi.org/10.1038/s41380-025-03033-w>
- Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Archives of Medical Science : AMS*, 11(6), 1164. <https://doi.org/10.5114/AOMS.2015.56342>
- Benwell, C. S. Y., Learmonth, G., Miniussi, C., Harvey, M., & Thut, G. (2015). Non-linear effects of transcranial direct current stimulation as a function of individual baseline performance: Evidence from biparietal tDCS influence on lateralized attention bias. *Cortex*, 69, 152–165. <https://doi.org/10.1016/J.CORTEX.2015.05.007>
- Bergfeld, I. O., Mantione, M., Figee, M., Schuurman, P. R., Lok, A., & Denys, D. (2018). Treatment-resistant depression and suicidality. *Journal of Affective Disorders*, 235, 362–367. <https://doi.org/10.1016/j.jad.2018.04.016>

- Boruah, S., Paskoff, G. R., Shender, B. S., Subit, D. L., Salzar, R. S., & Crandall, J. R. (2015). Variation of bone layer thicknesses and trabecular volume fraction in the adult male human calvarium. *Bone*, 77, 120–134. <https://doi.org/10.1016/j.bone.2015.04.031>
- Boschloo, L., Hieronymus, F., Lisinski, A., Cuijpers, P., & Eriksson, E. (2023). The complex clinical response to selective serotonin reuptake inhibitors in depression: a network perspective. *Translational Psychiatry* 2023 13:1, 13(1), 19-. <https://doi.org/10.1038/s41398-022-02285-2>
- Boutet, A., Gwun, D., Gramer, R., Ranjan, M., Elias, G. J. B., Tilden, D., Huang, Y., Li, S. X., Davidson, B., Lu, H., Tyrrell, P., Jones, R. M., Fasano, A., Hynynen, K., Kucharczyk, W., Schwartz, M. L., & Lozano, A. M. (2020a). The relevance of skull density ratio in selecting candidates for transcranial MR-guided focused ultrasound. *Journal of Neurosurgery*, 132(6), 1785–1791. <https://doi.org/10.3171/2019.2.JNS182571>
- Boutet, A., Gwun, D., Gramer, R., Ranjan, M., Elias, G. J. B., Tilden, D., Huang, Y., Li, S. X., Davidson, B., Lu, H., Tyrrell, P., Jones, R. M., Fasano, A., Hynynen, K., Kucharczyk, W., Schwartz, M. L., & Lozano, A. M. (2020b). The relevance of skull density ratio in selecting candidates for transcranial MR-guided focused ultrasound. *Journal of Neurosurgery*, 132(6), 1785–1791. <https://doi.org/10.3171/2019.2.JNS182571>
- Braun, V., Blackmore, J., Cleveland, R. O., & Butler, C. R. (2020). Transcranial ultrasound stimulation in humans is associated with an auditory confound that can be effectively masked. *Brain Stimulation*, 13(6), 1527–1534. <https://doi.org/10.1016/j.brs.2020.08.014>
- Brekhovskikh, L. M., & Godin, O. A. (1990). *Acoustics of Layered Media I* (Vol. 5). Springer Berlin Heidelberg. <https://doi.org/10.1007/978-3-642-52369-4>
- Brinker, S. T., Preiswerk, F., White, P. J., Mariano, T. Y., McDannold, N. J., & Bubrick, E. J. (2020). Focused Ultrasound Platform for Investigating Therapeutic Neuromodulation Across the Human Hippocampus. *Ultrasound in Medicine & Biology*, 46(5), 1270–1274. <https://doi.org/10.1016/j.ultrasmedbio.2020.01.007>
- Butler, C. R., Rhodes, E., Blackmore, J., Cheng, X., Peach, R. L., Veldsman, M., Sheerin, F., & Cleveland, R. O. (2022). Transcranial ultrasound stimulation to human middle temporal complex improves visual motion detection and modulates electrophysiological responses. *Brain Stimulation*, 15(5), 1236–1245. <https://doi.org/10.1016/j.brs.2022.08.022>
- Cai, H., Xie, X. M., Zhang, Q., Cui, X., Lin, J. X., Sim, K., Ungvari, G. S., Zhang, L., & Xiang, Y. T. (2021). Prevalence of Suicidality in Major Depressive Disorder: A Systematic Review and Meta-Analysis of Comparative Studies. *Frontiers in Psychiatry*, 12, 690130. <https://doi.org/10.3389/FPSYT.2021.690130/FULL>
- Cain, J. A., Visagan, S., Johnson, M. A., Crone, J., Blades, R., Spivak, N. M., Shattuck, D. W., & Monti, M. M. (2021). Real time and delayed effects of subcortical low

- intensity focused ultrasound. *Scientific Reports* 2021 11:1, 11(1), 6100-. <https://doi.org/10.1038/s41598-021-85504-y>
- Carreno, F. R., & Frazer, A. (2017). Vagal Nerve Stimulation for Treatment-Resistant Depression. *Neurotherapeutics*, 14(3), 716. <https://doi.org/10.1007/S13311-017-0537-8>
- Chaffai, S., Peyrin, F., Nuzzo, S., Porcher, R., Berger, G., & Laugier, P. (2002). Ultrasonic characterization of human cancellous bone using transmission and backscatter measurements: relationships to density and microstructure. *Bone*, 30(1), 229–237. [https://doi.org/10.1016/S8756-3282\(01\)00650-0](https://doi.org/10.1016/S8756-3282(01)00650-0)
- Chaffai, S., Padilla, F., Berger, G., & Laugier, P. (2000). In vitro measurement of the frequency-dependent attenuation in cancellous bone between 0.2 and 2 MHz. *The Journal of the Acoustical Society of America*, 108(3 Pt 1), 1281–1289. <https://doi.org/10.1121/1.1288934>
- Chang, W. S., Jung, H. H., Zadicario, E., Rachmilevitch, I., Tlusty, T., Vitek, S., & Chang, J. W. (2016). Factors associated with successful magnetic resonance-guided focused ultrasound treatment: efficiency of acoustic energy delivery through the skull. *Journal of Neurosurgery*, 124(2), 411–416. <https://doi.org/10.3171/2015.3.JNS142592>
- Chen, M., Peng, C., Wu, H., Huang, C. C., Kim, T., Traylor, Z., Muller, M., Chhatbar, P. Y., Nam, C. S., Feng, W., & Jiang, X. (2023). Numerical and experimental evaluation of low-intensity transcranial focused ultrasound wave propagation using human skulls for brain neuromodulation. *Medical Physics*, 50(1), 38–49. <https://doi.org/10.1002/mp.16090>
- Chen, S. G., Tsai, C. H., Lin, C. J., Lee, C. C., Yu, H. Y., Hsieh, T. H., & Liu, H. L. (2020). Transcranial focused ultrasound pulsation suppresses pentylenetetrazol induced epilepsy in vivo. *Brain Stimulation*, 13(1), 35–46. <https://doi.org/10.1016/j.brs.2019.09.011>
- Clement, G. T., & Hynynen, K. (2002). Micro-receiver guided transcranial beam steering. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, 49(4), 447–453. <https://doi.org/10.1109/58.996562>
- Clement, G. T., White, P. J., & Hynynen, K. (2004). Enhanced ultrasound transmission through the human skull using shear mode conversion. *The Journal of the Acoustical Society of America*, 115(3), 1356–1364. <https://doi.org/10.1121/1.1645610>
- Collins, C. J., Andriotis, O. G., Nedelkovski, V., Frank, M., Katsamenis, O. L., & Thurner, P. J. (2019). Bone Micro- and Nanomechanics. *Encyclopedia of Biomedical Engineering*, 1–3, 22–44. <https://doi.org/10.1016/B978-0-12-801238-3.99937-9>
- Connor, C. W., & Hynynen, K. (2004). Patterns of thermal deposition in the skull during transcranial focused ultrasound surgery. *IEEE Transactions on Biomedical Engineering*, 51(10), 1693–1706. <https://doi.org/10.1109/TBME.2004.831516>

- Cui, L., Li, S., Wang, S., Wu, X., Liu, Y., Yu, W., Wang, Y., Tang, Y., Xia, M., & Li, B. (2024). Major depressive disorder: hypothesis, mechanism, prevention and treatment. *Signal Transduction and Targeted Therapy* 2024 9:1, 9(1), 1–32. <https://doi.org/10.1038/s41392-024-01738-y>
- Cunningham, J. T., Mifflin, S. W., Gould, G. G., & Frazer, A. (2007). Induction of c-Fos and Δ FosB Immunoreactivity in Rat Brain by Vagal Nerve Stimulation. *Neuropsychopharmacology* 2008 33:8, 33(8), 1884–1895. <https://doi.org/10.1038/sj.npp.1301570>
- Dallapiazza, R. F., Timbie, K. F., Holmberg, S., Gatesman, J., Lopes, M. B., Price, R. J., Miller, G. W., Elias, W. J., Dallapiazza, R. F., Timbie, K. F., Holmberg, S., Gatesman, J., Lopes, M. B., Price, R. J., Miller, G. W., & Elias, W. J. (2017). Noninvasive neuromodulation and thalamic mapping with low-intensity focused ultrasound. *Journal of Neurosurgery*, 128(3), 875–884. <https://doi.org/10.3171/2016.11.JNS16976>
- Daniels, D., Sharabi, S., Last, D., Guez, D., Salomon, S., Zivli, Z., Castel, D., Volovick, A., Grinfeld, J., Rachmilevich, I., Amar, T., Liraz-Zaltsman, S., Sargsyan, N., Mardor, Y., & Harnof, S. (2018). Focused Ultrasound-Induced Suppression of Auditory Evoked Potentials in Vivo. *Ultrasound in Medicine & Biology*, 44(5), 1022–1030. <https://doi.org/10.1016/j.ultrasmedbio.2018.01.010>
- Darrow, D. P., O'Brien, P., Richner, T. J., Netoff, T. I., & Ebbini, E. S. (2019). Reversible neuroinhibition by focused ultrasound is mediated by a thermal mechanism. *Brain Stimulation*, 12(6), 1439. <https://doi.org/10.1016/j.brs.2019.07.015>
- Deffieux, T., Younan, Y., Wattiez, N., Tanter, M., Pouget, P., & Aubry, J. F. (2013). Low-Intensity Focused Ultrasound Modulates Monkey Visuomotor Behavior. *Current Biology*, 23(23), 2430–2433. <https://doi.org/10.1016/j.cub.2013.10.029>
- Deng, Z. De, Lisanby, S. H., & Peterchev, A. V. (2011). Electric field strength and focality in electroconvulsive therapy and magnetic seizure therapy: A finite element simulation study. *Journal of Neural Engineering*, 8(1), 016007. <https://doi.org/10.1088/1741-2560/8/1/016007>
- Deng, Z. De, Robins, P. L., Regenold, W., Rohde, P., Dannhauer, M., & Lisanby, S. H. (2023). How electroconvulsive therapy works in the treatment of depression: is it the seizure, the electricity, or both? *Neuropsychopharmacology* 2023 49:1, 49(1), 150–162. <https://doi.org/10.1038/s41386-023-01677-2>
- Di Biase, L., Falato, E., & Di Lazzaro, V. (2019). Transcranial Focused Ultrasound (tFUS) and Transcranial Unfocused Ultrasound (tUS) Neuromodulation: From Theoretical Principles to Stimulation Practices. *Frontiers in Neurology*, 10(JUN), 549. <https://doi.org/10.3389/FNEUR.2019.00549>
- D'Souza, M., Chen, K. S., Rosenberg, J., Elias, W. J., Eisenberg, H. M., Gwinn, R., Taira, T., Chang, J. W., Lipsman, N., Krishna, V., Igase, K., Yamada, K., Kishima, H., Cosgrove, R., Rumià, J., Kaplitt, M. G., Hirabayashi, H., Nandi, D., Henderson, J. M., ... Ghanouni, P. (2020a). Impact of skull density ratio on efficacy and safety

- of magnetic resonance-guided focused ultrasound treatment of essential tremor. *Journal of Neurosurgery*, 132(5), 1392–1397. <https://doi.org/10.3171/2019.2.JNS183517>
- D’Souza, M., Chen, K. S., Rosenberg, J., Elias, W. J., Eisenberg, H. M., Gwinn, R., Taira, T., Chang, J. W., Lipsman, N., Krishna, V., Igase, K., Yamada, K., Kishima, H., Cosgrove, R., Rumià, J., Kaplitt, M. G., Hirabayashi, H., Nandi, D., Henderson, J. M., ... Ghanouni, P. (2020b). Impact of skull density ratio on efficacy and safety of magnetic resonance-guided focused ultrasound treatment of essential tremor. *Journal of Neurosurgery*, 132(5), 1392–1397. <https://doi.org/10.3171/2019.2.JNS183517>
- Duck, F. (2013). *Physical properties of tissues: a comprehensive reference book*. Academic press.
- Elias, W. J., Lipsman, N., Ondo, W. G., Ghanouni, P., Kim, Y. G., Lee, W., Schwartz, M., Hynynen, K., Lozano, A. M., Shah, B. B., Huss, D., Dallapiazza, R. F., Gwinn, R., Witt, J., Ro, S., Eisenberg, H. M., Fishman, P. S., Gandhi, D., Halpern, C. H., ... Chang, J. W. (2016). A Randomized Trial of Focused Ultrasound Thalamotomy for Essential Tremor. *New England Journal of Medicine*, 375(8), 730–739. <https://doi.org/10.1056/nejmoa1600159>
- Enoka, R. M., Amiridis, I. G., & Duchateau, J. (2019). Electrical Stimulation of Muscle: Electrophysiology and Rehabilitation. <https://doi.org/10.1152/Physiol.00015.2019>, 35(1), 40–56. <https://doi.org/10.1152/PHYSIOL.00015.2019>
- Erhart, J., Pùlpán, P., & Pustka, M. (2017). *Piezoelectric Ceramic Resonators. Topics in Mining, Metallurgy and Materials Engineering*. <https://doi.org/10.1007/978-3-319-42481-1>
- Erturk, A., & Inman, D. J. (2011). Appendix A: Piezoelectric Constitutive Equations. *Piezoelectric Energy Harvesting*, 343–348. <https://doi.org/10.1002/9781119991151.APP1>
- Estermann, S. J., Förster-Streffleur, S., Hirtler, L., Streicher, J., Pahr, D. H., & Reisinger, A. (2021). Comparison of Thiel preserved, fresh human, and animal liver tissue in terms of mechanical properties. *Annals of Anatomy*, 236. <https://doi.org/10.1016/j.aanat.2021.151717>
- Feigin, V. L., Vos, T., Nichols, E., Owolabi, M. O., Carroll, W. M., Dichgans, M., Deuschl, G., Parmar, P., Brainin, M., & Murray, C. (2019). The global burden of neurological disorders: translating evidence into policy. *The Lancet. Neurology*, 19(3), 255. [https://doi.org/10.1016/S1474-4422\(19\)30411-9](https://doi.org/10.1016/S1474-4422(19)30411-9)
- Fisher, J. A. N., & Gumenchuk, I. (2018). Low-intensity focused ultrasound alters the latency and spatial patterns of sensory-evoked cortical responses in vivo. *Journal of Neural Engineering*, 15(3), 035004. <https://doi.org/10.1088/1741-2552/aaee1>
- Folloni, D., Verhagen, L., Mars, R. B., Fouragnan, E., Constans, C., Aubry, J. F., Rushworth, M. F. S., & Sallet, J. (2019). Manipulation of Subcortical and Deep

- Cortical Activity in the Primate Brain Using Transcranial Focused Ultrasound Stimulation. *Neuron*, 101(6), 1109-1116.e5. <https://doi.org/10.1016/j.neuron.2019.01.019>
- Fomenko, A., Chen, K. H. S., Nankoo, J. F., Saravanamuttu, J., Wang, Y., El-Baba, M., Xia, X., Seerala, S. S., Hynynen, K., Lozano, A. M., & Chen, R. (2020). Systematic examination of low-intensity ultrasound parameters on human motor cortex excitability and behaviour. *ELife*, 9, 1–68. <https://doi.org/10.7554/eLife.54497>
- Forster, A., Rodrigues, J., Ziebell, P., Sanguinetti, J. L., Allen, J. J., & Hewig, J. (2023). Investigating the role of the right inferior frontal gyrus in control perception: A double-blind cross-over study using ultrasonic neuromodulation. *Neuropsychologia*, 187(4), 108589. <https://doi.org/10.1016/j.neuropsychologia.2023.108589>
- Fry, F. J., & Barger, J. E. (1978). Acoustical properties of the human skull. *J Acoust Soc Am*, 63(5), 1576–1590. <https://doi.org/10.1121/1.381852>
- Fry, W. J., Wulff, V. J., Tucker, D., & Fry, F. J. (1950). Physical Factors Involved in Ultrasonically Induced Changes in Living Systems: I. Identification of Non-Temperature Effects. *The Journal of the Acoustical Society of America*, 22(6), 867–876. <https://doi.org/10.1121/1.1906707>
- Gagliardo, C., Midiri, M., Cannella, R., Napoli, A., Wragg, P., Collura, G., Marrale, M., Vincenzo Bartolotta, T., Catalano, C., & Lagalla, R. (2018). Transcranial magnetic resonance-guided focused ultrasound surgery at 1.5T: a technical note. *The Neuroradiology Journal*, 32(2), 132. <https://doi.org/10.1177/1971400918818743>
- Gao, P., Sun, Y., Zhang, G., Li, C., & Wang, L. (2023). A transducer positioning method for transcranial focused ultrasound treatment of brain tumors. *Frontiers in Neuroscience*, 17, 1277906. <https://doi.org/10.3389/FNINS.2023.1277906>
- Gibson, B. C., Sanguinetti, J. L., Badran, B. W., Yu, A. B., Klein, E. P., Abbott, C. C., Hansberger, J. T., & Clark, V. P. (2018). Increased excitability induced in the primary motor cortex by transcranial ultrasound stimulation. *Frontiers in Neurology*, 9(NOV), 429138. <https://doi.org/10.3389/fneur.2018.01007>
- Gimeno, L. A., Martin, E., Wright, O., & Treeby, B. E. (2019). Experimental Assessment of Skull Aberration and Transmission Loss at 270 kHz for Focused Ultrasound Stimulation of the Primary Visual Cortex. *IEEE International Ultrasonics Symposium, IUS*, 2019-October, 556–559. <https://doi.org/10.1109/ULTSYM.2019.8926097>
- Glasser, M. F., Coalson, T. S., Robinson, E. C., Hacker, C. D., Harwell, J., Yacoub, E., Ugurbil, K., Andersson, J., Beckmann, C. F., Jenkinson, M., Smith, S. M., & Van Essen, D. C. (2016). A multi-modal parcellation of human cerebral cortex. *Nature* 2016 536:7615, 536(7615), 171–178. <https://doi.org/10.1038/nature18933>
- Gonzalez, J., Morales, I. S., Villarreal, D. M., & Derrick, B. E. (2013). Low-frequency stimulation induces long-term depression and slow onset long-term potentiation at

- perforant path-dentate gyrus synapses in vivo. *Journal of Neurophysiology*, 111(6), 1259. <https://doi.org/10.1152/JN.00941.2012>
- Grimm, S., Ernst, J., Boesiger, P., Schuepbach, D., Hell, D., Boeker, H., & Northoff, G. (2009). Increased self-focus in major depressive disorder is related to neural abnormalities in subcortical-cortical midline structures. *Human Brain Mapping*, 30(8), 2617–2627. <https://doi.org/10.1002/HBM.20693>;JOURNAL:JOURNAL:10970193;WGROU P:STRING:PUBLICATION
- Guerra, A., Vicenzini, E., Cioffi, E., Colella, D., Cannavacciuolo, A., Pozzi, S., Caccia, B., Paparella, G., Di Stefano, G., Berardelli, A., & Bologna, M. (2021). Effects of transcranial ultrasound stimulation on trigeminal blink reflex excitability. *Brain Sciences*, 11(5), 645. <https://doi.org/10.3390/brainsci11050645>
- Guo, J., Song, X., Chen, X., Xu, M., & Ming, D. (2022a). Mathematical Model of Ultrasound Attenuation With Skull Thickness for Transcranial-Focused Ultrasound. *Frontiers in Neuroscience*, 15. <https://doi.org/10.3389/fnins.2021.778616>
- Guo, J., Song, X., Chen, X., Xu, M., & Ming, D. (2022b). Mathematical Model of Ultrasound Attenuation With Skull Thickness for Transcranial-Focused Ultrasound. *Frontiers in Neuroscience*, 15. <https://doi.org/10.3389/fnins.2021.778616>
- Hameroff, S., Trakas, M., Duffield, C., Annabi, E., Gerace, M. B., Boyle, P., Lucas, A., Amos, Q., Buadu, A., & Badal, J. J. (2013). Transcranial ultrasound (TUS) effects on mental states: A pilot study. *Brain Stimulation*, 6(3), 409–415. <https://doi.org/10.1016/j.brs.2012.05.002>
- Hayner, M., & Hynynen, K. (2001). Numerical analysis of ultrasonic transmission and absorption of oblique plane waves through the human skull. *The Journal of the Acoustical Society of America*, 110(6), 3319–3330. <https://doi.org/10.1121/1.1410964>
- Heimbuch, I. S., Fan, T. K., Wu, A. D., Faas, G. C., Charles, A. C., & Iacoboni, M. (2022). Ultrasound stimulation of the motor cortex during tonic muscle contraction. *PLOS ONE*, 17(4), e0267268. <https://doi.org/10.1371/journal.pone.0267268>
- Hodgkin, A. L., & Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *The Journal of Physiology*, 117(4), 500. <https://doi.org/10.1113/JPHYSIOL.1952.SP004764>
- Holmes, S. E., Scheinost, D., Finnema, S. J., Naganawa, M., Davis, M. T., DellaGioia, N., Nabulsi, N., Matuskey, D., Angarita, G. A., Pietrzak, R. H., Duman, R. S., Sanacora, G., Krystal, J. H., Carson, R. E., & Esterlis, I. (2019). Lower synaptic density is associated with depression severity and network alterations. *Nature Communications* 2019 10:1, 10(1), 1–10. <https://doi.org/10.1038/s41467-019-09562-7>

- Hosseini, S., Puonti, O., Treeby, B., Hanson, L. G., & Thielscher, A. (2023). A head template for computational dose modelling for transcranial focused ultrasound stimulation. *NeuroImage*, 277, 120227. <https://doi.org/10.1016/J.NEUROIMAGE.2023.120227>
- Human Tissue (Scotland) Act 2006*. (n.d.).
- Husain, M. M., Rush, A. J., Fink, M., Knapp, R., Petrides, G., Rummans, T., Biggs, M. M., O'Connor, K., Rasmussen, K., Little, M., Zhao, W., Bernstein, H. J., Smith, G., Mueller, M., McClintock, S. M., Bailine, S. H., & Kellner, C. H. (2004). Speed of response and remission in major depressive disorder with acute electroconvulsive therapy (ECT): a Consortium for Research in ECT (CORE) report. *The Journal of Clinical Psychiatry*, 65(4), 485–491. <https://doi.org/10.4088/JCP.V65N0406>
- IEEE Standard on Piezoelectricity. (1987). <https://doi.org/10.1109/IEEESTD.1988.79638>
- Jakes, P., Erdem, E., Eichel, R. A., Jin, L., & Damjanovic, D. (2011). Position of defects with respect to domain walls in Fe³⁺-doped Pb[Zr_{0.52}Ti_{0.48}]O₃ piezoelectric ceramics. *Applied Physics Letters*, 98(7). <https://doi.org/10.1063/1.3555465>
- Jeong, H., Im, J. J., Park, J. S., Na, S. H., Lee, W., Yoo, S. S., Song, I. U., & Chung, Y. A. (2021). A pilot clinical study of low-intensity transcranial focused ultrasound in Alzheimer's disease. *Ultrasonography (Seoul, Korea)*, 40(4), 512–519. <https://doi.org/10.14366/USG.20138>
- Jeong, H., Song, I. U., Chung, Y. A., Park, J. S., Na, S. H., Im, J. J., Bikson, M., Lee, W., & Yoo, S. S. (2022). Short-Term Efficacy of Transcranial Focused Ultrasound to the Hippocampus in Alzheimer's Disease: A Preliminary Study. *Journal of Personalized Medicine* 2022, Vol. 12, Page 250, 12(2), 250. <https://doi.org/10.3390/jpm12020250>
- Jin, C., Moore, D., Snell, J., & Paeng, D.-G. (2020). An open-source phase correction toolkit for transcranial focused ultrasound. *BMC Biomedical Engineering*, 2(1), 9. <https://doi.org/10.1186/S42490-020-00043-3>
- Johnson, K. A., Okun, M. S., Scangos, K. W., Mayberg, H. S., & de Hemptinne, C. (2024). Deep brain stimulation for refractory major depressive disorder: a comprehensive review. *Molecular Psychiatry* 2024 29:4, 29(4), 1075–1087. <https://doi.org/10.1038/s41380-023-02394-4>
- Johnstone, A., Nandi, T., Martin, E., Bestmann, S., Stagg, C., & Treeby, B. (2021). A range of pulses commonly used for human transcranial ultrasound stimulation are clearly audible. *Brain Stimulation*, 14(5), 1353–1355. <https://doi.org/10.1016/j.brs.2021.08.015>
- Jung, N. Y., Rachmilevitch, I., Sibiger, O., Amar, T., Zadicario, E., & Chang, J. W. (2019). Factors Related to Successful Energy Transmission of Focused Ultrasound through a Skull : A Study in Human Cadavers and Its Comparison with Clinical Experiences. *Journal of Korean Neurosurgical Society*, 62(6), 712. <https://doi.org/10.3340/jkns.2018.0226>

- Kaiser, R. H., Andrews-Hanna, J. R., Wager, T. D., & Pizzagalli, D. A. (2015). Large-scale network dysfunction in Major Depressive Disorder: Meta-analysis of resting-state functional connectivity. *JAMA Psychiatry*, 72(6), 603. <https://doi.org/10.1001/JAMAPSYCHIATRY.2015.0071>
- Khachaturian, Z., Boutellier, J.-M. C., Damborsky, J., & Khachaturian, A. S. (2025). Perspective on “Brain Network Disorders.” *Brain Network Disorders*, 1(1), 3–6. <https://doi.org/10.1016/J.BND.2024.12.002>
- Khairova, R. A., MacHado-Vieira, R., Du, J., & Manji, H. K. (2009). A potential role for pro-inflammatory cytokines in regulating synaptic plasticity in major depressive disorder. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, 12(4), 561. <https://doi.org/10.1017/S1461145709009924>
- Kim, H. C., Lee, W., Kowsari, K., Weisholtz, D. S., & Yoo, S. S. (2022). Effects of focused ultrasound pulse duration on stimulating cortical and subcortical motor circuits in awake sheep. *PLOS ONE*, 17(12), e0278865. <https://doi.org/10.1371/journal.pone.0278865>
- Kim, H. C., Lee, W., Weisholtz, D. S., & Yoo, S. S. (2023). Transcranial focused ultrasound stimulation of cortical and thalamic somatosensory areas in human. *PLOS ONE*, 18(7), e0288654. <https://doi.org/10.1371/journal.pone.0288654>
- Kim, H., Chiu, A., Lee, S. D., Fischer, K., & Yoo, S. S. (2014). Focused Ultrasound-mediated Non-invasive Brain Stimulation: Examination of Sonication Parameters. *Brain Stimulation*, 7(5), 748–756. <https://doi.org/10.1016/j.brs.2014.06.011>
- Kim, H., Park, M. Y., Lee, S. D., Lee, W., Chiu, A., & Yoo, S. S. (2015). Suppression of EEG visual-evoked potentials in rats through neuromodulatory focused ultrasound. *Neuroreport*, 26(4), 211–215. <https://doi.org/10.1097/WNR.0000000000000330>
- Kim, S., Jo, Y., Kook, G., Pasquinelli, C., Kim, H., Kim, K., Hoe, H. S., Choe, Y., Rhim, H., Thielscher, A., Kim, J., & Lee, H. J. (2021). Transcranial focused ultrasound stimulation with high spatial resolution. *Brain Stimulation*, 14(2), 290–300. <https://doi.org/10.1016/j.brs.2021.01.002>
- Kim, Y. G., Kim, S. E., Lee, J., Hwang, S., Yoo, S. S., & Lee, H. W. (2022). Neuromodulation Using Transcranial Focused Ultrasound on the Bilateral Medial Prefrontal Cortex. *Journal of Clinical Medicine* 2022, Vol. 11, Page 3809, 11(13), 3809. <https://doi.org/10.3390/jcm11133809>
- Kirchner, T., Villringer, C., & Laufer, J. (2023). Evaluation of ultrasound sensors for transcranial photoacoustic sensing and imaging. *Photoacoustics*, 33, 100556. <https://doi.org/10.1016/j.pacs.2023.100556>
- Klomjai, W., Katz, R., & Lackmy-Vallée, A. (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Annals of Physical and*

- Rehabilitation Medicine*, 58(4), 208–213.
<https://doi.org/10.1016/J.REHAB.2015.05.005>
- Kolibius, L. D., Roux, F., Parish, G., Ter Wal, M., Van Der Plas, M., Chelvarajah, R., Sawlani, V., Rollings, D. T., Lang, J. D., Gollwitzer, S., Walther, K., Hopfengärtner, R., Kreiselmeier, G., Hamer, H., Staresina, B. P., Wimber, M., Bowman, H., & Hanslmayr, S. (2023). Hippocampal neurons code individual episodic memories in humans. *Nature Human Behaviour* 2023 7:11, 7(11), 1968–1979. <https://doi.org/10.1038/s41562-023-01706-6>
- Kosnoff, J., Gonsisko, C., Yu, K., Zhang, J., Ding, Y., Zhang, Y., & He, B. (2026). Transcranial focused ultrasound induces source localizable cortical activation in resting state humans when applied concurrently with transcranial electric stimulation. *Nature Communications* 2026 17:1, 17(1), 2023-. <https://doi.org/10.1038/s41467-026-69853-8>
- Kosnoff, J., Yu, K., Liu, C., & He, B. (2024). Transcranial focused ultrasound to V5 enhances human visual motion brain-computer interface by modulating feature-based attention. *Nature Communications* 2024 15:1, 15(1), 1–18. <https://doi.org/10.1038/s41467-024-48576-8>
- Krames, E. S., Peckham, P. H., & Rezai, A. R. (2018). Neuromodulation: Comprehensive Textbook of Principles, Technologies, and Therapies. *Neuromodulation: Comprehensive Textbook of Principles, Technologies, and Therapies, Second Edition: Volume 1-3*, 1–3, 1–1764. <https://doi.org/10.1016/C2015-0-04712-8>
- Krokhmal, A., Simcock, I. C., Treeby, B. E., & Martin, E. (2025). A comparative study of experimental and simulated ultrasound beam propagation through cranial bones. *Physics in Medicine and Biology*, 70(2), 025007. <https://doi.org/10.1088/1361-6560/ADA19D>
- Küçükibrahimoğlu, E., Saygın, M. Z., Çalışkan, M., Kaplan, O. K., Ünsal, C., & Gören, M. Z. (2009). The change in plasma GABA, glutamine and glutamate levels in fluoxetine- Or S-citalopram-treated female patients with major depression. *European Journal of Clinical Pharmacology*, 65(6), 571–577. <https://doi.org/10.1007/S00228-009-0650-7/FIGURES/3>
- Kuhn, T., Spivak, N. M., Dang, B. H., Becerra, S., Halavi, S. E., Rotstein, N., Rosenberg, B. M., Hiller, S., Swenson, A., Cvijanovic, L., Dang, N., Sun, M., Kronemyer, D., Berlow, R., Revett, M. R., Suthana, N., Monti, M. M., & Bookheimer, S. (2023). Transcranial focused ultrasound selectively increases perfusion and modulates functional connectivity of deep brain regions in humans. *Frontiers in Neural Circuits*, 17, 1120410. <https://doi.org/10.3389/fncir.2023.1120410>
- Kung, C. (2005). A possible unifying principle for mechanosensation. *Nature* 2005 436:7051, 436(7051), 647–654. <https://doi.org/10.1038/nature03896>
- Lee, J., Paeng, D.-G., & Ha, K. (2020). Attenuation of the human skull at broadband frequencies by using a carbon nanotube composite photoacoustic transducer. *The*

- Journal of the Acoustical Society of America*, 148(3), 1121–1129. <https://doi.org/10.1121/10.0001791>
- Lee, W., Chung, Y. A., Jung, Y., Song, I. U., & Yoo, S. S. (2016). Simultaneous acoustic stimulation of human primary and secondary somatosensory cortices using transcranial focused ultrasound. *BMC Neuroscience* 2016 17:1, 17(1), 68-. <https://doi.org/10.1186/s12868-016-0303-6>
- Lee, W., Croce, P., Margolin, R. W., Cammalleri, A., Yoon, K., & Yoo, S. S. (2018). Transcranial focused ultrasound stimulation of motor cortical areas in freely-moving awake rats. *BMC Neuroscience* 2018 19:1, 19(1), 57-. <https://doi.org/10.1186/s12868-018-0459-3>
- Lee, W. H., Lisanby, S. H., Laine, A. F., & Peterchev, A. V. (2016). Comparison of electric field strength and spatial distribution of electroconvulsive therapy and magnetic seizure therapy in a realistic human head model. *European Psychiatry*, 36, 55–64. <https://doi.org/10.1016/J.EURPSY.2016.03.003>
- Lee, W., Kim, H. C., Jung, Y., Chung, Y. A., Song, I. U., Lee, J. H., & Yoo, S. S. (2016). Transcranial focused ultrasound stimulation of human primary visual cortex. *Scientific Reports*, 6. <https://doi.org/10.1038/srep34026>
- Lee, W., Kim, H., Jung, Y., Song, I. U., Chung, Y. A., & Yoo, S. S. (2015a). Image-guided transcranial focused ultrasound stimulates human primary somatosensory cortex. *Scientific Reports*, 5. <https://doi.org/10.1038/srep08743>
- Lee, W., Kim, H., Jung, Y., Song, I. U., Chung, Y. A., & Yoo, S. S. (2015b). Image-Guided Transcranial Focused Ultrasound Stimulates Human Primary Somatosensory Cortex. *Scientific Reports*, 5, 8743. <https://doi.org/10.1038/SREP08743>
- Legon, W., Ai, L., Bansal, P., & Mueller, J. K. (2018a). Neuromodulation with single-element transcranial focused ultrasound in human thalamus. *Human Brain Mapping*, 39(5), 1995–2006. <https://doi.org/10.1002/hbm.23981>
- Legon, W., Ai, L., Bansal, P., & Mueller, J. K. (2018b). Neuromodulation with single-element transcranial focused ultrasound in human thalamus. *Human Brain Mapping*, 39(5), 1995–2006. <https://doi.org/10.1002/HBM.23981>
- Legon, W., Bansal, P., Tyshynsky, R., Ai, L., & Mueller, J. K. (2018). Transcranial focused ultrasound neuromodulation of the human primary motor cortex. *Scientific Reports*, 8(1). <https://doi.org/10.1038/s41598-018-28320-1>
- Legon, W., Sato, T. F., Opitz, A., Mueller, J., Barbour, A., Williams, A., & Tyler, W. J. (2014). Transcranial focused ultrasound modulates the activity of primary somatosensory cortex in humans. *Nature Neuroscience* 2013 17:2, 17(2), 322–329. <https://doi.org/10.1038/nn.3620>
- Leung, S. A., Moore, D., Webb, T. D., Snell, J., Ghanouni, P., & Butts Pauly, K. (2021). Transcranial focused ultrasound phase correction using the hybrid angular

- spectrum method. *Scientific Reports* 2021 11:1, 11(1), 6532-.
<https://doi.org/10.1038/s41598-021-85535-5>
- Li, G., Qiu, W., Zhang, Z., Jiang, Q., Su, M., Cai, R., Li, Y., Cai, F., Deng, Z., Xu, D., Zhang, H., & Zheng, H. (2019). Noninvasive Ultrasonic Neuromodulation in Freely Moving Mice. *IEEE Transactions on Biomedical Engineering*, 66(1), 217–224. <https://doi.org/10.1109/TBME.2018.2821201>
- Liebe, T., Li, M., Colic, L., Munk, M. H. J., Sweeney-Reed, C. M., Woelfer, M., Kretschmar, M. A., Steiner, J., von Düring, F., Behnisch, G., Schott, B. H., & Walter, M. (2018). Ketamine influences the locus coeruleus norepinephrine network, with a dependency on norepinephrine transporter genotype – a placebo controlled fMRI study. *NeuroImage: Clinical*, 20, 715–723. <https://doi.org/10.1016/j.nicl.2018.09.001>
- Lillie, E. M., Urban, J. E., Lynch, S. K., Weaver, A. A., & Stitzel, J. D. (2016). Evaluation of Skull Cortical Thickness Changes with Age and Sex from Computed Tomography Scans. *Journal of Bone and Mineral Research*, 31(2), 299–307. <https://doi.org/10.1002/JBMR.2613>;REQUESTEDJOURNAL:JOURNAL:15234681;WGROU:STRING:PUBLICATION
- Liu, A., Vöröslakos, M., Kronberg, G., Henin, S., Krause, M. R., Huang, Y., Opitz, A., Mehta, A., Pack, C. C., Krekelberg, B., Berényi, A., Parra, L. C., Melloni, L., Devinsky, O., & Buzsáki, G. (2018). Immediate neurophysiological effects of transcranial electrical stimulation. *Nature Communications* 2018 9:1, 9(1), 5092-.
<https://doi.org/10.1038/s41467-018-07233-7>
- Liu, C., Yu, K., Niu, X., & He, B. (2021). Transcranial Focused Ultrasound Enhances Sensory Discrimination Capability through Somatosensory Cortical Excitation. *Ultrasound in Medicine & Biology*, 47(5), 1356–1366. <https://doi.org/10.1016/j.ultrasmedbio.2021.01.025>
- Lu, N., Hall, T. L., Sukovich, J. R., Choi, S. W., Snell, J., McDannold, N., & Xu, Z. (2022). Two-step Aberration Correction: application to Transcranial Histotripsy. *Physics in Medicine and Biology*, 67(12), 10.1088/1361-6560/ac72ed. <https://doi.org/10.1088/1361-6560/AC72ED>
- Mahoney, J. J., Thompson-Lake, D. G. Y., Ranjan, M., Marton, J. L., Carpenter, J. S., Zheng, W., Berry, J. H., Farmer, D. L., D’Haese, P., Finomore, V. S., Tirumalai, P., Mears, A. S., Suffridge, J., Ames, A., Hodder, S. L., & Rezai, A. R. (2023). Low-Intensity Focused Ultrasound Targeting the Bilateral Nucleus Accumbens as a Potential Treatment for Substance Use Disorder: A First-in-Human Report. *Biological Psychiatry*, 94(11), e41–e43. <https://doi.org/10.1016/j.biopsych.2023.06.031>
- Malone, D. A., Dougherty, D. D., Rezai, A. R., Carpenter, L. L., Friehs, G. M., Eskandar, E. N., Rauch, S. L., Rasmussen, S. A., Machado, A. G., Kubu, C. S., Tyrka, A. R., Price, L. H., Stypulkowski, P. H., Giftakis, J. E., Rise, M. T., Malloy, P. F., Salloway, S. P., & Greenberg, B. D. (2009). Deep Brain Stimulation of the Ventral

- Capsule/Ventral Striatum for Treatment-Resistant Depression. *Biological Psychiatry*, 65(4), 267–275. <https://doi.org/10.1016/j.biopsych.2008.08.029>
- Manta, S., Dong, J., Debonnel, G., & Blier, P. (2009). Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *Journal of Psychiatry & Neuroscience: JPN*, 34(4), 272. <https://doi.org/10.1139/jpn.0936>
- Marsac, L., Chauvet, D., La Greca, R., Boch, A. L., Chaumoitre, K., Tanter, M., & Aubry, J. F. (2017a). Ex vivo optimisation of a heterogeneous speed of sound model of the human skull for non-invasive transcranial focused ultrasound at 1 MHz. *International Journal of Hyperthermia*, 33(6), 635–645. <https://doi.org/10.1080/02656736.2017.1295322>;ISSUE:ISSUE:DOI
- Marsac, L., Chauvet, D., La Greca, R., Boch, A. L., Chaumoitre, K., Tanter, M., & Aubry, J. F. (2017b). Ex vivo optimisation of a heterogeneous speed of sound model of the human skull for non-invasive transcranial focused ultrasound at 1 MHz. *International Journal of Hyperthermia*, 33(6), 635–645. <https://doi.org/10.1080/02656736.2017.1295322>
- Martin, E., Roberts, M., Grigoras, I. F., Wright, O., Nandi, T., Rieger, S. W., Campbell, J., den Boer, T., Cox, B. T., Stagg, C. J., & Treeby, B. E. (2025a). Ultrasound system for precise neuromodulation of human deep brain circuits. *Nature Communications* 2025 16:1, 16(1), 8024-. <https://doi.org/10.1038/s41467-025-63020-1>
- Martin, E., Roberts, M., Grigoras, I. F., Wright, O., Nandi, T., Rieger, S. W., Campbell, J., den Boer, T., Cox, B. T., Stagg, C. J., & Treeby, B. E. (2025b). Ultrasound system for precise neuromodulation of human deep brain circuits. *Nature Communications* 2025 16:1, 16(1), 8024-. <https://doi.org/10.1038/s41467-025-63020-1>
- Martin, S. J., Grimwood, P. D., & Morris, R. G. M. (2000). Synaptic plasticity and memory: An evaluation of the hypothesis. *Annual Review of Neuroscience*, 23(Volume 23, 2000), 649–711. <https://doi.org/10.1146/ANNUREV.NEURO.23.1.649/CITE/REFWORKS>
- McDevitt, R. A., Tiran-Cappello, A., Shen, H., Balderas, I., Britt, J. P., Marino, R. A. M., Chung, S. L., Richie, C. T., Harvey, B. K., & Bonci, A. (2014). Serotonergic versus Nonserotonergic Dorsal Raphe Projection Neurons: Differential Participation in Reward Circuitry. *Cell Reports*, 8(6), 1857–1869. <https://doi.org/10.1016/J.CELREP.2014.08.037>
- McIntyre, R. S., Alsuwaidan, M., Baune, B. T., Berk, M., Demyttenaere, K., Goldberg, J. F., Gorwood, P., Ho, R., Kasper, S., Kennedy, S. H., Ly-Uson, J., Mansur, R. B., McAllister-Williams, R. H., Murrough, J. W., Nemeroff, C. B., Nierenberg, A. A., Rosenblat, J. D., Sanacora, G., Schatzberg, A. F., ... Maj, M. (2023). Treatment-resistant depression: definition, prevalence, detection, management, and investigational interventions. *World Psychiatry*, 22(3), 394. <https://doi.org/10.1002/WPS.21120>

- Min, B. K., Bystritsky, A., Jung, K. I., Fischer, K., Zhang, Y., Maeng, L. S., In Park, S., Chung, Y. A., Jolesz, F. A., & Yoo, S. S. (2011). Focused ultrasound-mediated suppression of chemically-induced acute epileptic EEG activity. *BMC Neuroscience*, 12(1), 23-. <https://doi.org/10.1186/1471-2202-12-23>
- Mohammadjavadi, M., Ash, R. T., Glover, G. H., & Pauly, K. B. (2025). Optimization of MR acoustic radiation force imaging (MR-ARFI) for human transcranial focused ultrasound. *Magnetic Resonance in Medicine*, 94(3), 1060–1071. <https://doi.org/10.1002/mrm.30539>
- Moncrieff, J., Cooper, R. E., Stockmann, T., Amendola, S., Hengartner, M. P., & Horowitz, M. A. (2022). The serotonin theory of depression: a systematic umbrella review of the evidence. *Molecular Psychiatry* 2022 28:8, 28(8), 3243–3256. <https://doi.org/10.1038/s41380-022-01661-0>
- Monte-Silva, K., Kuo, M. F., Hesselthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., & Nitsche, M. A. (2013). Induction of Late LTP-Like Plasticity in the Human Motor Cortex by Repeated Non-Invasive Brain Stimulation. *Brain Stimulation*, 6(3), 424–432. <https://doi.org/10.1016/J.BRS.2012.04.011>
- Morriss, R., Briley, P. M., Webster, L., Abdelghani, M., Barber, S., Bates, P., Brookes, C., Hall, B., Ingram, L., Kurkar, M., Lankappa, S., Liddle, P. F., McAllister-Williams, R. H., O’Neil-Kerr, A., Pszczolkowski, S., Suazo Di Paola, A., Walters, Y., & Auer, D. P. (2024). Connectivity-guided intermittent theta burst versus repetitive transcranial magnetic stimulation for treatment-resistant depression: a randomized controlled trial. *Nature Medicine* 2024 30:2, 30(2), 403–413. <https://doi.org/10.1038/s41591-023-02764-z>
- Mueller, J., Legon, W., Opitz, A., Sato, T. F., & Tyler, W. J. (2014). Transcranial Focused Ultrasound Modulates Intrinsic and Evoked EEG Dynamics. *Brain Stimulation*, 7(6), 900–908. <https://doi.org/10.1016/j.brs.2014.08.008>
- Murphy, K. R., Nandi, T., Kop, B., Osada, T., N’Djin, W. A., Lueckel, M., Caulfield, K. A., Fomenko, A., Siebner, H. R., Ugawa, Y., Verhagen, L., Bestmann, S., Martin, E., Pauly, K. B., Fouragnan, E., & Bergmann, T. O. (2025). A practical guide to transcranial ultrasonic stimulation from the IFCN-endorsed ITRUSST consortium. *Clinical Neurophysiology*, 171, 192–226. <https://doi.org/10.1016/J.CLINPH.2025.01.004>
- Naftchi-Ardebili, K., Menz, M. D., Salahshoor, H., Popelka, G. R., Baccus, S. A., & Butts Pauly, K. (2024). Focal Volume, Acoustic Radiation Force, and Strain in Two-Transducer Regimes. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, 71(10), 1199. <https://doi.org/10.1109/TUFFC.2024.3456048>
- Nakajima, K., Osada, T., Ogawa, A., Tanaka, M., Oka, S., Kamagata, K., Aoki, S., Oshima, Y., Tanaka, S., & Konishi, S. (2022). A causal role of anterior prefrontal-putamen circuit for response inhibition revealed by transcranial ultrasound stimulation in humans. *Cell Reports*, 40(7), 111197. <https://doi.org/10.1016/j.celrep.2022.111197>

- Neves, G., Cooke, S. F., & Bliss, T. V. P. (2008). Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. *Nature Reviews Neuroscience* 2008 9:1, 9(1), 65–75. <https://doi.org/10.1038/nrn2303>
- Niu, X., Yu, K., & He, B. (2022). Transcranial focused ultrasound induces sustained synaptic plasticity in rat hippocampus. *Brain Stimulation*, 15(2), 352–359. <https://doi.org/10.1016/j.brs.2022.01.015>
- O’Neil, H. T. (1949). Theory of Focusing Radiators. *The Journal of the Acoustical Society of America*, 21(5), 516–526. <https://doi.org/10.1121/1.1906542>
- O’Reardon, J. P., Cristancho, P., & Peshek, A. D. (2006). Vagus Nerve Stimulation (VNS) and Treatment of Depression: To the Brainstem and Beyond. *Psychiatry (Edgmont)*, 3(5), 54. <https://pmc.ncbi.nlm.nih.gov/articles/PMC2990624/>
- Ousdal, O. T., Brancati, G. E., Kessler, U., Erchinger, V., Dale, A. M., Abbott, C., & Oltedal, L. (2022). The Neurobiological Effects of Electroconvulsive Therapy Studied Through Magnetic Resonance: What Have We Learned, and Where Do We Go? *Biological Psychiatry*, 91(6), 540–549. <https://doi.org/10.1016/J.BIOPSYCH.2021.05.023>
- Park, T. Y., Pahk, K. J., & Kim, H. (2019). Method to optimize the placement of a single-element transducer for transcranial focused ultrasound. *Computer Methods and Programs in Biomedicine*, 179, 104982. <https://doi.org/10.1016/J.CMPB.2019.104982>
- Pichardo, S., Sin, V. W., & Hynynen, K. (2011). Multi-frequency characterization of the speed of sound and attenuation coefficient for longitudinal transmission of freshly excised human skulls. *Physics in Medicine and Biology*, 56(1), 219–250. <https://doi.org/10.1088/0031-9155/56/1/014>
- Pinton, G., Aubry, J. F., Bossy, E., Muller, M., Pernot, M., & Tanter, M. (2012a). Attenuation, scattering, and absorption of ultrasound in the skull bone. *Medical Physics*, 39(1), 299–307. <https://doi.org/10.1118/1.3668316>
- Pinton, G., Aubry, J. F., Bossy, E., Muller, M., Pernot, M., & Tanter, M. (2012b). Attenuation, scattering, and absorption of ultrasound in the skull bone. *Medical Physics*, 39(1), 299–307. <https://doi.org/10.1118/1.3668316>
- Pinton, G., Aubry, J. F., Fink, M., & Tanter, M. (2011). Effects of nonlinear ultrasound propagation on high intensity brain therapy. *Medical Physics*, 38(3), 1207–1216. <https://doi.org/10.1118/1.3531553>
- Qiu, Z. (2014). *DOCTOR OF PHILOSOPHY Development of MRI-compatible Transducer Array for Focused Ultrasound Surgery: The Use of Relaxor-based Piezocrystals.*
- Quesseveur, G., David, D. J., Gaillard, M. C., Pla, P., Wu, M. V., Nguyen, H. T., Nicolas, V., Auregan, G., David, I., Dranovsky, A., Hantraye, P., Hen, R., Gardier, A. M., Déglon, N., & Guiard, B. P. (2013). BDNF overexpression in mouse hippocampal astrocytes promotes local neurogenesis and elicits anxiolytic-like activities.

- Translational Psychiatry* 2013 3:4, 3(4), e253–e253. <https://doi.org/10.1038/tp.2013.30>
- Ramachandran, S., Gao, H., Yttri, E., Yu, K., & He, B. (2025). Parameter-dependent cell-type specific effects of transcranial focused ultrasound stimulation in an awake head-fixed rodent model. *Journal of Neural Engineering*, 22(2), 026022. <https://doi.org/10.1088/1741-2552/ADBB1F>
- Ramasubbu, R., Golding, S., Williams, K., Mackie, A., Macqueen, G., & Kiss, Z. H. T. (2021). Recruitment Challenges for Studies of Deep Brain Stimulation for Treatment-Resistant Depression. *Neuropsychiatric Disease and Treatment*, 17, 765. <https://doi.org/10.2147/NDT.S299913>
- Ren, L., Zhai, Z., Xiang, Q., Zhuo, K., Zhang, S., Zhang, Y., Jiao, X., Tong, S., Liu, D., & Sun, J. (2023). Transcranial ultrasound stimulation modulates the interhemispheric balance of excitability in human motor cortex. *Journal of Neural Engineering*, 20(1), 016043. <https://doi.org/10.1088/1741-2552/acb50d>
- Reznik, S. J., Sanguinetti, J. L., Tyler, W. J., Daft, C., & Allen, J. J. B. (2020). A double-blind pilot study of transcranial ultrasound (TUS) as a five-day intervention: TUS mitigates worry among depressed participants. *Neurology, Psychiatry and Brain Research*, 37, 60–66. <https://doi.org/10.1016/j.npbr.2020.06.004>
- Rhee, T. G., Shim, S. R., Forester, B. P., Nierenberg, A. A., McIntyre, R. S., Papakostas, G. I., Krystal, J. H., Sanacora, G., & Wilkinson, S. T. (2022). Efficacy and Safety of Ketamine vs Electroconvulsive Therapy Among Patients With Major Depressive Episode: A Systematic Review and Meta-analysis. *JAMA Psychiatry*, 79(12), 1162–1172. <https://doi.org/10.1001/JAMAPSYCHIATRY.2022.3352>
- Riis, T. S., Webb, T. D., & Kubanek, J. (2022). Acoustic properties across the human skull. *Ultrasonics*, 119. <https://doi.org/10.1016/j.ultras.2021.106591>
- Sackeim, H. A., Aaronson, S. T., Carpenter, L. L., Hutton, T. M., Mina, M., Pages, K., Verdoliva, S., & West, W. S. (2020). Clinical outcomes in a large registry of patients with major depressive disorder treated with Transcranial Magnetic Stimulation. *Journal of Affective Disorders*, 277, 65–74. <https://doi.org/10.1016/J.JAD.2020.08.005>
- Sammartino, F., Beam, D. W., Snell, J., & Krishna, V. (2019). Kranion, an open-source environment for planning transcranial focused ultrasound surgery: technical note. *Journal of Neurosurgery*, 132(4), 1249–1255. <https://doi.org/10.3171/2018.11.JNS181995>
- Sanguinetti, J. L., Hameroff, S., Smith, E. E., Sato, T., Daft, C. M. W., Tyler, W. J., & Allen, J. J. B. (2020a). Transcranial Focused Ultrasound to the Right Prefrontal Cortex Improves Mood and Alters Functional Connectivity in Humans. *Frontiers in Human Neuroscience*, 14, 494085. <https://doi.org/10.3389/fnhum.2020.00052>
- Sanguinetti, J. L., Hameroff, S., Smith, E. E., Sato, T., Daft, C. M. W., Tyler, W. J., & Allen, J. J. B. (2020b). Transcranial Focused Ultrasound to the Right Prefrontal

- Cortex Improves Mood and Alters Functional Connectivity in Humans. *Frontiers in Human Neuroscience*, 14. <https://doi.org/10.3389/fnhum.2020.00052>
- Sapozhnikov, O. A., Tsysar, S. A., Khokhlova, V. A., & Kreider, W. (2015). Acoustic holography as a metrological tool for characterizing medical ultrasound sources and fields. *The Journal of the Acoustical Society of America*, 138(3), 1515–1532. <https://doi.org/10.1121/1.4928396>
- Sarica, C., Nankoo, J. F., Fomenko, A., Grippe, T. C., Yamamoto, K., Samuel, N., Milano, V., Vetkas, A., Darmani, G., Cizmeci, M. N., Lozano, A. M., & Chen, R. (2022). Multi-modal investigation of transcranial ultrasound-induced neuroplasticity of the human motor cortex. *Brain Stimulation*, 15(6), 1337–1347. <https://doi.org/10.1016/j.brs.2022.05.002>
- Schimek, N., Burke-Conte, Z., Abernethy, J., Schimek, M., Burke-Conte, C., Bobola, M., Stocco, A., & Mourad, P. D. (2020). Repeated Application of Transcranial Diagnostic Ultrasound Towards the Visual Cortex Induced Illusory Visual Percepts in Healthy Participants. *Frontiers in Human Neuroscience*, 14, 66. <https://doi.org/10.3389/fnhum.2020.00066>
- Schneider, U., Pedroni, E., & Lomax, A. (1996). The calibration of CT Hounsfield units for radiotherapy treatment planning. In *Phys. Med. Biol* (Vol. 41). <http://iopscience.iop.org/0031-9155/41/1/009>
- Semkovska, M., & McLoughlin, D. M. (2010). Objective Cognitive Performance Associated with Electroconvulsive Therapy for Depression: A Systematic Review and Meta-Analysis. *Biological Psychiatry*, 68(6), 568–577. <https://doi.org/10.1016/J.BIOPSYCH.2010.06.009>
- Shamli Oghli, Y., Grippe, T., Arora, T., Hoque, T., Darmani, G., & Chen, R. (2023). Mechanisms of theta burst transcranial ultrasound induced plasticity in the human motor cortex. *Brain Stimulation*, 16(4), 1135–1143. <https://doi.org/10.1016/j.brs.2023.07.056>
- Shimokawa, H., Shindo, T., Ishiki, A., Tomita, N., Ichijyo, S., Watanabe, T., Nakata, T., Eguchi, K., Kikuchi, Y., Shioto, T., Takahashi, J., Yasuda, S., & Arai, H. (2022). A Pilot Study of Whole-Brain Low-Intensity Pulsed Ultrasound Therapy for Early Stage of Alzheimer's Disease (LIPUS-AD): A Randomized, Double-Blind, Placebo-Controlled Trial. *The Tohoku Journal of Experimental Medicine*, 258(3), 167–175. <https://doi.org/10.1620/tjem.2022.J078>
- Shin, D. H., Son, S., & Kim, E. Y. (2023). Low-Energy Transcranial Navigation-Guided Focused Ultrasound for Neuropathic Pain: An Exploratory Study. *Brain Sciences*, 13(10), 1433. <https://doi.org/10.3390/brainsci13101433>
- Short, B., Fong, J., Galvez, V., Shelker, W., & Loo, C. K. (2018). Side-effects associated with ketamine use in depression: a systematic review. *The Lancet Psychiatry*, 5(1), 65–78. [https://doi.org/10.1016/S2215-0366\(17\)30272-9](https://doi.org/10.1016/S2215-0366(17)30272-9)
- Siebner, H. R., Funke, K., Aberra, A. S., Antal, A., Bestmann, S., Chen, R., Classen, J., Davare, M., Di Lazzaro, V., Fox, P. T., Hallett, M., Karabanov, A. N., Kesselheim,

- J., Beck, M. M., Koch, G., Liebetanz, D., Meunier, S., Miniussi, C., Paulus, W., ... Ugawa, Y. (2022). Transcranial magnetic stimulation of the brain: What is stimulated? – A consensus and critical position paper. *Clinical Neurophysiology*, 140, 59–97. <https://doi.org/10.1016/J.CLINPH.2022.04.022>
- Smart, O., Choi, K. S., Riva-Posse, P., Tiruvadi, V., Rajendra, J., Waters, A. C., Crowell, A. L., Edwards, J., Gross, R. E., & Mayberg, H. S. (2018). Initial Unilateral Exposure to Deep Brain Stimulation in Treatment-Resistant Depression Patients Alters Spectral Power in the Subcallosal Cingulate. *Frontiers in Computational Neuroscience*, 12. <https://doi.org/10.3389/FNCOM.2018.00043>
- Strohman, A., Payne, B., In, A., Stebbins, K., & Legon, W. (2024). Low-Intensity Focused Ultrasound to the Human Dorsal Anterior Cingulate Attenuates Acute Pain Perception and Autonomic Responses. *Journal of Neuroscience*, 44(8). <https://doi.org/10.1523/JNEUROSCI.1011-23.2023>
- Taghizadeh, S., Labuda, C., & Mobley, J. (2018). Development of a Tissue-Mimicking Phantom of the Brain for Ultrasonic Studies. *Ultrasound in Medicine and Biology*, 44(12), 2813–2820. <https://doi.org/10.1016/j.ultrasmedbio.2018.08.012>
- Tang, S. C., & Clement, G. T. (2010). Standing-wave suppression for transcranial ultrasound by random modulation. *IEEE Transactions on Biomedical Engineering*, 57(1), 203–205. <https://doi.org/10.1109/TBME.2009.2028653>
- The impact of CT image parameters and skull heterogeneity modeling on the accuracy of transcranial focused ultrasound simulations.* (2021). <https://doi.org/10.1088/1741-2552/abf68d>
- Treeby, B. E., Cox, ; B T, & Cox, B. T. (2014). Modeling power law absorption and dispersion in viscoelastic solids using a split-field and the fractional Laplacian. *The Journal of the Acoustical Society of America*, 136(4), 1499–1510. <https://doi.org/10.1121/1.4894790>
- Treeby, B. E., & Cox, B. T. (2010). k-Wave: MATLAB toolbox for the simulation and reconstruction of photoacoustic wave fields. *Journal of Biomedical Optics*, 15(2), 021314. <https://doi.org/10.1117/1.3360308>
- Tyler, W. J., Tufail, Y., Finsterwald, M., Tauchmann, M. L., Olson, E. J., & Majestic, C. (2008). Remote Excitation of Neuronal Circuits Using Low-Intensity, Low-Frequency Ultrasound. *PLOS ONE*, 3(10), e3511. <https://doi.org/10.1371/journal.pone.0003511>
- Voineskos, D., Daskalakis, Z. J., & Blumberger, D. M. (2020). Management of Treatment-Resistant Depression: Challenges and Strategies. *Neuropsychiatric Disease and Treatment*, 16, 221. <https://doi.org/10.2147/NDT.S198774>
- Wang, N., Li, H., Liao, J., Halliwell, T., & Huang, Z. (2026). Predicting transcranial ultrasound insertion loss using skull CT: A deep learning approach. *Ultrasonics*, 163, 107976. <https://doi.org/10.1016/J.ULTRAS.2026.107976>

- Wattiez, N., Constans, C., Deffieux, T., Daye, P. M., Tanter, M., Aubry, J. F., & Pouget, P. (2017). Transcranial ultrasonic stimulation modulates single-neuron discharge in macaques performing an antisaccade task. *Brain Stimulation*, *10*(6), 1024–1031. <https://doi.org/10.1016/j.brs.2017.07.007>
- Watts, W. W., Clennell, B., Jiang, J. K., Izaki-Lee, K., Binodh, A., Cuthell, R., Tonyali, D., Crompton, J., Taaffe, R., Alqahtani, A., Andrieu, A., Besinga, I. R., Heesom, K., Steward, T. G. J., Cho, K., Drinkwater, B. W., Molnár, E., & Whitcomb, D. J. (2025). Brief transcranial focused ultrasound stimulation causes lasting modifications to the synaptic circuitry of the hippocampus. *Brain Stimulation*, *18*(5), 1587–1599. <https://doi.org/10.1016/J.BRS.2025.08.014>
- White, D. N., Curry, G. R., & Stevenson, R. J. (1978). THE ACOUSTIC CHARACTERISTICS OF THE SKULL*. In *Ultrasound in Med, & Biol* (Vol. 4). Pergamon Press.
- White, P. J., Clement, G. T., & Hynynen, K. (2006a). Local frequency dependence in transcranial ultrasound transmission. *Phys Med Biol*, *51*(9), 2293–2305.
- White, P. J., Clement, G. T., & Hynynen, K. (2006b). Longitudinal and shear mode ultrasound propagation in human skull bone. *Ultrasound Med Biol*, *32*(7), 1085–1096.
- Woodham, R. D., Selvaraj, S., Lajmi, N., Hobday, H., Sheehan, G., Ghazi-Noori, A. R., Lagerberg, P. J., Rizvi, M., Kwon, S. S., Orhii, P., Maislin, D., Hernandez, L., Machado-Vieira, R., Soares, J. C., Young, A. H., & Fu, C. H. Y. (2025). Home-based transcranial direct current stimulation treatment for major depressive disorder: a fully remote phase 2 randomized sham-controlled trial. *Nature Medicine*, *31*(1), 87–95. <https://doi.org/10.1038/S41591-024-03305-Y>;SUBJMETA
- World Health Organization. (2017). *Depression and other common mental disorders: global health estimates*. <https://iris.who.int/handle/10665/254610>
- Wray, N. R., Ripke, S., Mattheisen, M., Trzaskowski, M., Byrne, E. M., Abdellaoui, A., Adams, M. J., Agerbo, E., Air, T. M., Andlauer, T. M. F., Bacanu, S. A., Bækvad-Hansen, M., Beekman, A. F. T., Bigdeli, T. B., Binder, E. B., Blackwood, D. R. H., Bryois, J., Buttenschøn, H. N., Bybjerg-Grauholm, J., ... Sullivan, P. F. (2018). Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nature Genetics* *2018* *50*:5, *50*(5), 668–681. <https://doi.org/10.1038/s41588-018-0090-3>
- Xia, X., Fomenko, A., Nankoo, J. F., Zeng, K., Wang, Y., Zhang, J., Lozano, A. M., & Chen, R. (2021). Time course of the effects of low-intensity transcranial ultrasound on the excitability of ipsilateral and contralateral human primary motor cortex. *NeuroImage*, *243*. <https://doi.org/10.1016/j.neuroimage.2021.118557>
- Xiao, C., Cen, C., Huang, Q., Xie, F., Liu, L., Lin, W., & Xiao, Y. (2025). Synthetic CT Generation from MRI Using Generative Adversarial Networks for MR-Only Brain Radiotherapy Planning. *Technology in Cancer Research & Treatment*, *24*, 15330338251400832. <https://doi.org/10.1177/15330338251400833>

- Yaakub, S. N., White, T. A., Roberts, J., Martin, E., Verhagen, L., Stagg, C. J., Hall, S., & Fouragnan, E. F. (2023). Transcranial focused ultrasound-mediated neurochemical and functional connectivity changes in deep cortical regions in humans. *Nature Communications*, 14(1). <https://doi.org/10.1038/s41467-023-40998-0>
- Yang, H., Yuan, Y., Wang, X., & Li, X. (2020). Closed-Loop Transcranial Ultrasound Stimulation for Real-Time Non-invasive Neuromodulation in vivo. *Frontiers in Neuroscience*, 14, 508153. <https://doi.org/10.3389/fnins.2020.00445>
- Yang, P. F., Phipps, M. A., Jonathan, S., Newton, A. T., Byun, N., Gore, J. C., Grissom, W. A., Caskey, C. F., & Chen, L. M. (2021). Bidirectional and state-dependent modulation of brain activity by transcranial focused ultrasound in non-human primates. *Brain Stimulation*, 14(2), 261–272. <https://doi.org/10.1016/j.brs.2021.01.006>
- Yao, J., Chen, C., Guo, Y., Yang, Y., Liu, X., Chu, S., Ai, Q., Zhang, Z., Lin, M., Yang, S., & Chen, N. (2023). A Review of Research on the Association between Neuron–Astrocyte Signaling Processes and Depressive Symptoms. *International Journal of Molecular Sciences*, 24(8), 6985. <https://doi.org/10.3390/IJMS24086985>
- Yoo, S., Mittelstein, D. R., Hurt, R. C., Lacroix, J., & Shapiro, M. G. (2022a). Focused ultrasound excites cortical neurons via mechanosensitive calcium accumulation and ion channel amplification. *Nature Communications*, 13(1). <https://doi.org/10.1038/s41467-022-28040-1>
- Yoo, S., Mittelstein, D. R., Hurt, R. C., Lacroix, J., & Shapiro, M. G. (2022b). Focused ultrasound excites cortical neurons via mechanosensitive calcium accumulation and ion channel amplification. *Nature Communications*, 13(1). <https://doi.org/10.1038/s41467-022-28040-1>
- Yoo, S. S., Kim, H., Min, B. K., Franck, E., & Park, S. (2011). Transcranial focused ultrasound to the thalamus alters anesthesia time in rats. *NeuroReport*, 22(15), 783–787. <https://doi.org/10.1097/WNR.0b013e32834b2957>
- Yoshimura, S., Okamoto, Y., Onoda, K., Matsunaga, M., Okada, G., Kunisato, Y., Yoshino, A., Ueda, K., Suzuki, S. ichi, & Yamawaki, S. (2014). Cognitive behavioral therapy for depression changes medial prefrontal and ventral anterior cingulate cortex activity associated with self-referential processing. *Social Cognitive and Affective Neuroscience*, 9(4), 487–493. <https://doi.org/10.1093/SCAN/NST009>
- Yu, K., Liu, C., Niu, X., & He, B. (2021). Transcranial Focused Ultrasound Neuromodulation of Voluntary Movement-Related Cortical Activity in Humans. *IEEE Transactions on Bio-Medical Engineering*, 68(6), 1923–1931. <https://doi.org/10.1109/TBME.2020.3030892>
- Yu, K., Niu, X., Krook-Magnuson, E., & He, B. (2021). Intrinsic functional neuron-type selectivity of transcranial focused ultrasound neuromodulation. *Nature Communications* 2021 12:1, 12(1), 2519-. <https://doi.org/10.1038/s41467-021-22743-7>

- Yu, K., Schmitt, S., Ni, Y., Crane, E. C., Smith, M. A., & He, B. (2024). Transcranial focused ultrasound remotely modulates extrastriate visual cortex by stimulating frontal eye field with subregion specificity. *Journal of Neural Engineering*, 21(6), 066018. <https://doi.org/10.1088/1741-2552/ad9406>
- Yuan, Y., Wang, Z., Liu, M., & Shoham, S. (2020). Cortical hemodynamic responses induced by low-intensity transcranial ultrasound stimulation of mouse cortex. *NeuroImage*, 211(7321), 116597. <https://doi.org/10.1016/j.neuroimage.2020.116597>
- Zanos, P., Moaddel, R., Morris, P. J., Georgiou, P., Fischell, J., Elmer, G. I., Alkondon, M., Yuan, P., Pribut, H. J., Singh, N. S., Dossou, K. S. S., Fang, Y., Huang, X. P., Mayo, C. L., Wainer, I. W., Albuquerque, E. X., Thompson, S. M., Thomas, C. J., Zarate, C. A., & Gould, T. D. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature* 2016 533:7604, 533(7604), 481–486. <https://doi.org/10.1038/nature17998>
- Zeng, K., Darmani, G., Fomenko, A., Xia, X., Tran, S., Nankoo, J. F., Shamli Oghli, Y., Wang, Y., Lozano, A. M., & Chen, R. (2022). Induction of Human Motor Cortex Plasticity by Theta Burst Transcranial Ultrasound Stimulation. *Annals of Neurology*, 91(2), 238–252. <https://doi.org/10.1002/ana.26294>
- Zeng, K., Li, Z., Xia, X., Wang, Z., Darmani, G., Li, X., & Chen, R. (2024a). Effects of different sonication parameters of theta burst transcranial ultrasound stimulation on human motor cortex. *Brain Stimulation*, 17(2), 258–268. <https://doi.org/10.1016/j.brs.2024.03.001>
- Zeng, K., Li, Z., Xia, X., Wang, Z., Darmani, G., Li, X., & Chen, R. (2024b). Effects of different sonication parameters of theta burst transcranial ultrasound stimulation on human motor cortex. *Brain Stimulation*, 17(2), 258–268. <https://doi.org/10.1016/j.brs.2024.03.001>
- Zhai, Z., Ren, L., Song, Z., Xiang, Q., Zhuo, K., Zhang, S., Li, X., Zhang, Y., Jiao, X., Tong, S., Sun, J., & Liu, D. (2023). The efficacy of low-intensity transcranial ultrasound stimulation on negative symptoms in schizophrenia: A double-blind, randomized sham-controlled study. *Brain Stimulation*, 16(3), 790–792. <https://doi.org/10.1016/j.brs.2023.04.021>
- Zhang, M. F., Chen, W. Z., Huang, F. B., Peng, Z. Y., Quan, Y. C., & Tang, Z. M. (2022). Low-intensity transcranial ultrasound stimulation facilitates hand motor function and cortical excitability: A crossover, randomized, double blind study. *Frontiers in Neurology*, 13, 926027. <https://doi.org/10.3389/fneur.2022.926027>
- Zhang, T., Guo, B., Zuo, Z., Long, X., Hu, S., Li, S., Su, X., Wang, Y., & Liu, C. (2023). Excitatory-inhibitory modulation of transcranial focus ultrasound stimulation on human motor cortex. *CNS Neuroscience and Therapeutics*, 29(12), 3829–3841. <https://doi.org/10.1111/cns.14303>
- Zhang, Y., Daday, C., Gu, R. X., Cox, C. D., Martinac, B., de Groot, B. L., & Walz, T. (2021). Visualization of the mechanosensitive ion channel MscS under membrane

- tension. *Nature* 2021 590:7846, 590(7846), 509–514. <https://doi.org/10.1038/s41586-021-03196-w>
- Zhang, Y., Ren, L., Liu, K., Tong, S., Yuan, T. F., & Sun, J. (2021). Transcranial ultrasound stimulation of the human motor cortex. *IScience*, 24(12), 103429. <https://doi.org/10.1016/j.isci.2021.103429>
- Zhou, X., Xiao, Q., Xie, L., Yang, F., Wang, L., & Tu, J. (2019). Astrocyte, a promising target for mood disorder interventions. *Frontiers in Molecular Neuroscience*, 12, 459366. <https://doi.org/10.3389/FNMOL.2019.00136/XML>
- Ziebell, P., Rodrigues, J., Forster, A., Sanguinetti, J. L., Allen, J. J., & Hewig, J. (2023). Inhibition of midfrontal theta with transcranial ultrasound explains greater approach versus withdrawal behavior in humans. *Brain Stimulation*, 16(5), 1278–1288. <https://doi.org/10.1016/j.brs.2023.08.011>
- Zou, J., Wang, Y. X., Dou, F. F., Lü, H. Z., Ma, Z. W., Lu, P. H., & Xu, X. M. (2010). Glutamine synthetase down-regulation reduces astrocyte protection against glutamate excitotoxicity to neurons. *Neurochemistry International*, 56(4), 577–584. <https://doi.org/10.1016/J.NEUINT.2009.12.021>