A novel image guided focused ultrasound platform for therapeutic delivery across the blood-brain barrier in mice

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1 Experimental Outline

Blood-brain barrier (BBB) penetration of systemically administered drugs is a significant challenge for the treatment of many neurological disorders and diseases of the brain. Focused ultrasound (FUS) therapy has shown efficacy against brain disease targets due to its ability to non-invasively and transiently disrupt the BBB.

FUS is applied external to the body, with or without injectable microbubble contrast agents, and exerts thermal and mechanical bioeffects by targeting ultrasound energy deposition in a specific area making it an ideal direct treatment or adjuvant treatment option. To limit off-target effects, an important component of FUS is the use of image guidance to delineate the treatment area and ensure that only a specific spatial region will be targeted.

Herein we present a modification to a commercially available robotic preclinical ultrasound system that allows for whole-body mouse imaging and image guidance for FUS delivery to user-defined targets. We evaluate its inter-user targeting accuracy in an acoustically responsive phantom material and demonstrate its ability to open the BBB in a mouse model, using ex vivo fluorescence imaging (FLI) for validation.

2 Imaging Methods

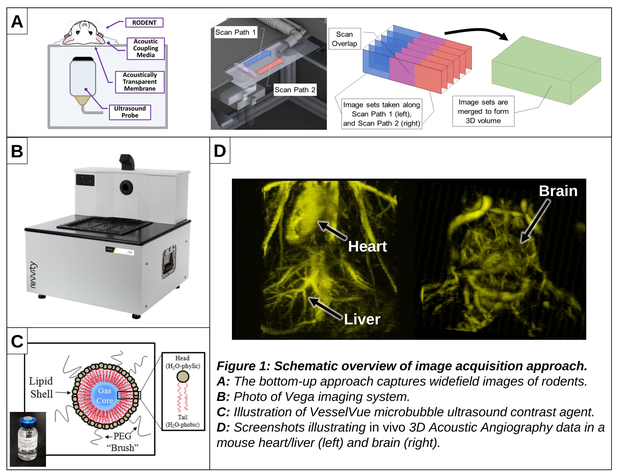


Figure 1: Schematic overview of image acquisition approach. ***A:*** *The bottom-up approach captures widefield images of rodents.* ***B:*** *Photo of Vega imaging system.* ***C:*** *Illustration of VesselVue microbubble ultrasound contrast agent.* ***D:*** *Screenshots illustrating* in vivo *3D Acoustic Angiography data in a mouse heart/liver (left) and brain (right).*

3 FUS Transducer Array Design

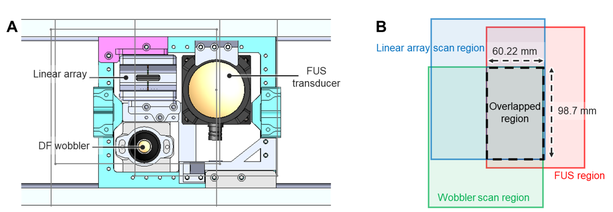


Figure 2: Transducer design. (A) Schematic of Vega imaging arrays with modified FUS addition. (B) Scan and FUS regions of array with overlap. Total combined array area is 60 x 100 mm.

4 FUS Targeting Validation

The FUS transducer array was tested using a custom grid phantom.

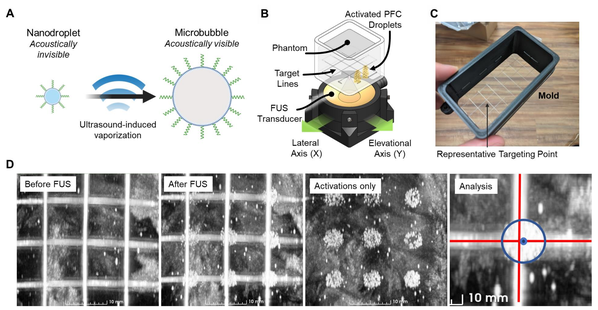


Figure 3: Overview of in vitro testing of FUS system. (A) A schematic representation of the nanodroplets used for the FUS experiments. (B) Schematic showing phantom placement on top of transducer for validation. (C) The polyvinyl alcohol phantom with grid pattern used to validate targeting accuracy of pressure-calibrated FUS array. (D) Images before and after FUS on phantom for quantification of targeting accuracy. The last two images show the size of treatment spots and a zoomed in view of an activation error. Phantom prepared as described in Durham et. al. Ultrasound in Medicine and Biology 2022.

5 FUS Targeting Accuracy

Operator & reader accuracy was tested for automated FUS system.

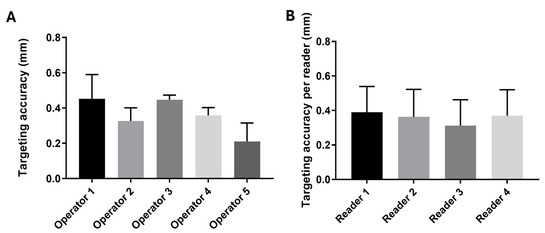


Figure 4. Validation of inter-user reproducibility. (A) 5 independent operators performing 3 FUS treatments each were evaluated for targeting accuracy. Variation was found to not be significant (p=0.34). (B) 15 FUS treatments per reader were evaluated for targeting accuracy. Variation was found to not be significant (p=0.28). Data are shown as mean ± standard deviation.

6 Ex vivo FUS Ablation

Localized heating from ablation was observed.

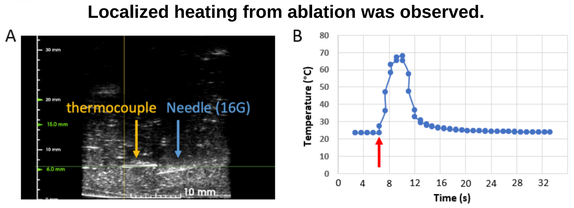
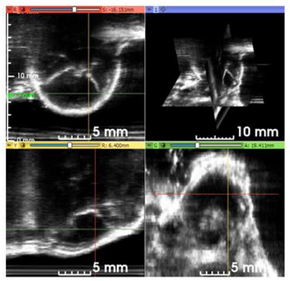


Figure 5: Evaluation of heat generation in chicken tissue. (A) B-mode US image of chicken tissue with thermocouple and needle labeled. (B) Temperature readings of thermocouple after FUS targeting on needle. Localized temperatures of tissue were found to exceed 65°C (1MHz, 3.0 MPa peak-negative-pressure, 200 Hz PRF, 2000 cycles, for a total duration of 2 s with the onset time shown by the red arrow).

7 Image-Guided FUS Workflow



Step 1: US Image Capture

Images of the tissue are taken on the Vega and the target locations for FUS application are identified.

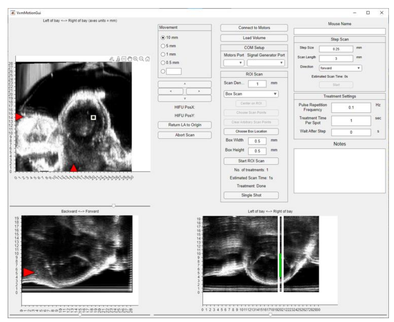
- Guidance can be with linear or wobbler arrays
- Linear array center frequency = 18 MHz
- Wobbler array center frequency = 26 MHz
- 40 mm imaging depth with linear array
- 100 µm resolution (@10 mm) with wobbler
- Acoustic angiography allows better definition in brain

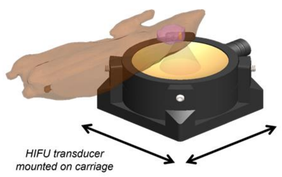
Step 2: FUS Treatment Plan

Images are loaded into a FUS treatment software package (written in MATLAB) and a treatment plan is created.

The user can define treatment spots or utilize a preset grid array. They can also use preset patterns/shapes.

Pulse repetition frequency (Hz), treatment time per spot (sec) and delay between treatment steps can be customized by the user.





Step 3: FUS Treatment

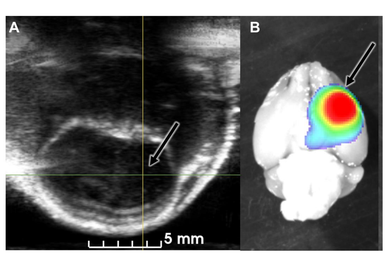
The treatment plan is then automatically administered using the Vega platform with the HIFU transducer.

- 1 MHz, 41.8 mm concave aperture transducer
- Geometric focus = 45 mm
- H-201-MR (Sonic Concepts, Bothell, WA, USA)
- Paired with impedance matching circuit (H201-02, Sonic Concepts)
- Matlab controlled arbitrary waveform generator (Tekronix AFG3021C) and amplifier

Step 4: Validation of BBB Disruption

Ex vivo fluorescent imaging was performed on excised brain tissue to confirm Evans blue deposition in the FUS target.

- 5-minute treatment @ 1 MHz (transmitted pressure of ~500 kPa), duty cycle of 10%, PRF 100Hz (1000 cycles per burst)
- Mice were allowed to wake and return to house for 3 hours before perfusion and harvest
- Image shows FUS treatment targeting (A) and Evans blue extravasation and fluorescence (B) at targeted area



8 FUS-Induced Immunomodulation

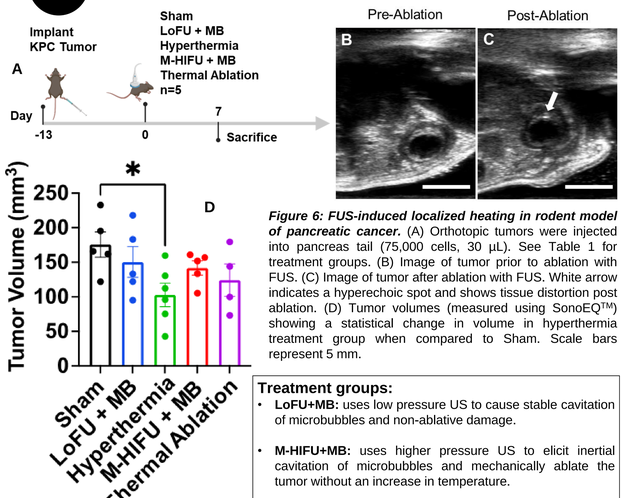


Figure 6: FUS-induced localized heating in rodent model of pancreatic cancer. (A) Orthotopic tumors were injected into pancreas tail (75,000 cells, 30 µL). See Table 1 for treatment groups. (B) Image of tumor prior to ablation with FUS. (C) Image of tumor after ablation with FUS. White arrow indicates a hyperechoic spot and shows tissue distortion post ablation. (D) Tumor volumes (measured using SonoEQ™) showing a statistical change in volume in hyperthermia treatment group when compared to Sham. Scale bars represent 5 mm.

Treatment groups:

- **LoFU+MB:** uses low pressure US to cause stable cavitation of microbubbles and non-ablative damage.
- **M-HIFU+MB:** uses higher pressure US to elicit inertial cavitation of microbubbles and mechanically ablate the tumor without an increase in temperature.
- **Hyperthermia:** utilizes the localized heat produced by FUS and heats the tumor to sub-lethal temperatures of 40-50°C.
- **Thermal Ablation:** heats the tumor to ablative temperatures of 65-80°C.

Treatment Group	Ultrasound parameters
Sham	Untreated control group (no ultrasound application)
LoFU + MB	MB dose: 3x10^8 MBs (infusion) PNP: 0.5 MPa; DC: 10%; I_{STPA}: 0.83 W/cm^2; TTT: 2-4 min (5 s/spot)
M-HIFU + MB	MB dose: 3x10^8 MBs (infusion) PNP: 6 MPa; DC: 0.002%; I_{STPA}: 0.024 W/cm^2; TTT: 3.5 min (20 s/spot)
Hyperthermia	40-50°C PNP: 3.7 MPa; DC: 30%; I_{STPA}: 137 W/cm^2; TTT: 2 min (1 spot)
Thermal Ablation	>65°C for 30 s PNP: 5 MPa; DC: 60%; I_{STPA}: 500 W/cm^2; TTT: 30 s (1 spot)

Table 1. Immunomodulatory treatment groups. *MB: microbubble; PNP: peak-negative-pressure; DC: duty cycle; ISTPA: derated spatial-peak temporal-average intensity; TTT: total treatment time*

9 Summary

By leveraging ultrasound guidance, this system provides a theranostic platform for precise FUS delivery with anatomical and functional imaging, in a user interface that facilitates custom treatment definitions and reduces ultrasound operator variability.

This unique combination is a significant first step in providing better tools for preclinical studies and lowering the barrier of entry for researchers to investigate novel therapeutic targets for neurological and other diseases.

Future studies will investigate real-time cavitation monitoring and feedback during treatments.

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