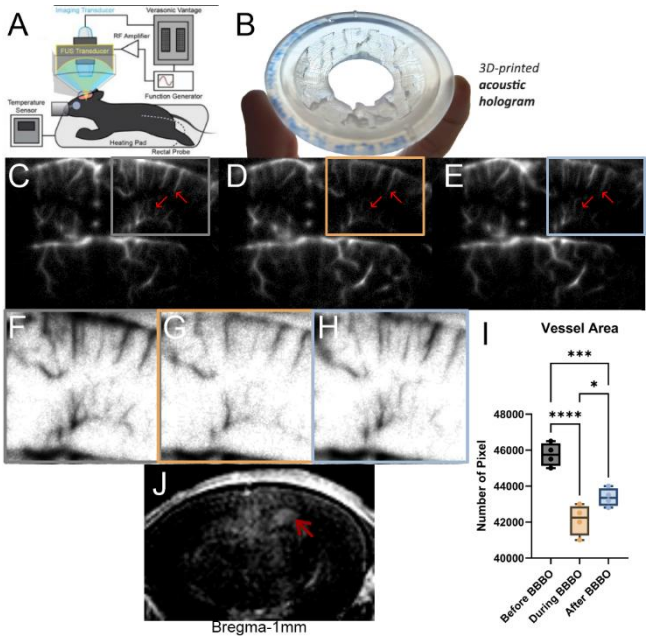
Transcranial Ultrasound Localization Microscopy for Monitoring Blood-Brain Barrier Opening in Mice

Background, Motivation and Objective

Focused ultrasound (FUS) combined with microbubbles allows transient, localized opening of the blood-brain barrier (BBBO) for targeted drug delivery. Monitoring BBBO is essential to ensure safety and efficacy, but conventional techniques rely on contrast-enhanced MRI or histopathology, which are either costly or not available for in-vivo monitoring. Ultrasound Localization Microscopy (ULM) offers non-invasive, high-resolution vascular imaging beyond the diffraction limit. Recently, [Bae et al., 2024] showed that 2D ULM can be used to confirm opening after FUS. In this study, we present transcranial 2D ULM imaging performed before, during, and after BBBO, enabled by a custom 3D-printed acoustic hologram that shifts the FUS focal spot closer to the transducer.

Statement of Contribution/Methods

All procedures were conducted following animal protocol approval. An acoustic hologram was designed and 3D-printed to shift the FUS focal spot from 68 mm to 22 mm closer to the FUS transducer. ULM imaging was performed with a linear array transducer (L22-15) placed transcranially to acquire ultrasound raw data at 1000 frame/s before, during, and after BBBO using in-house polydisperse microbubbles. Boluses of 25 µL/min were used before and after BBBO. For BBBO, a single bolus of 100 µL was injected. Clutter filtering was applied using SVD, and localized microbubbles were tracked using radial symmetry and Hungarian algorithm. The vascular density and vessel area were quantified throughout four mice (WT female 10 w). Contrast enhanced T1-weighted were performed post-treatment to confirm BBBO.



Results/Discussion Fig1 A,B illustrate the acoustic setup and the printed hologram used. Panels C–E show ULM density maps at baseline, during, and post-BBBO. Panels F–H highlight vessel density changes across timepoints, with noticeable vascular constriction during BBBO in the hippocampal vasculature. Quantification in Fig1I shows a statistically significant reduction in vessel cross-sectional diameter during BBBO followed by partial recovery. MRI validation is shown in Fig1J. The result demonstrates the capability of transcranial ULM to dynamically monitor vascular changes during BBBO, enabling real-time, non-invasive feedback. These findings provide new directions for integrating ULM into therapeutic ultrasound workflows for safer, more effective brain drug delivery.