This FUS Foundation project consists of 2 specific aims:

Specific Aim 1: To determine FUS pressure and MB diameter combinations for reversible opening of the BBB in intracranial rat brain tumors. We will define FUS pressure thresholds for safe and reversible BBB opening to an MR contrast agent (gadolinium) in invasive intracranial brain tumors (human U251 glioblastoma xenografts) as a function of monodisperse MB diameter.

Specific Aim 2: To determine optimal conditions for delivering brain penetrating nanoparticle (BPN) formulations. The pressure thresholds from Aim #1 will be used as boundaries in determining optimal FUS and MB parameters for delivering BPN formulations across the BBB to U251 tumors.

For the first 6 months of this project, our work was slowed considerably by technical difficulties with the small animal MR-guided focused ultrasound system. The system returned from the manufacturer several months after after having undergone a putative "upgrade", but we encountered issues with the x-y-z stage translation. These issues were eventually resolved, but we lost several months of productivity due to technical problems. The system is now operational and we are finally underway with tis project.

Blood-brain barrier opening with MR-guided focused ultrasound and microbubbles. Over the past few months, we have been working on validating the multiple target capacity of the FUS system. Sonicating at multiple targets within a single sonication period eliminates variations in dosing of therapeutics as well as contrast agent, enabling more precise animal-to-animal comparisons. Using our pulsing protocol (10 ms pulses every 2 seconds for a total of 120 seconds), we verified that we were able to sonicate up to 6 spots, separated by 3mm, in the rat brain (Figure 1). We were also able to demonstrate resolution as fine as 2 mm between spots (Figure 2).

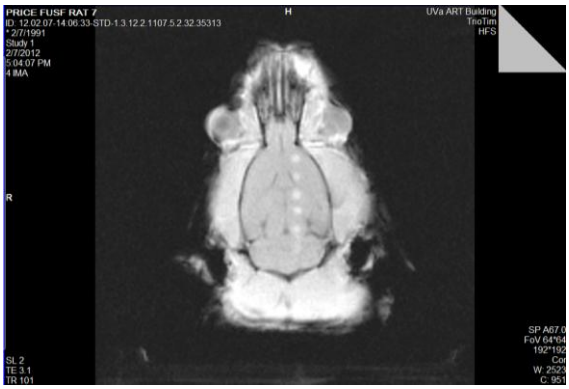

Figure 1: Six sonications, separated by 3 mm, are possible with our pulsing protocol. Sixth spot is faint, located in the cerebellum.

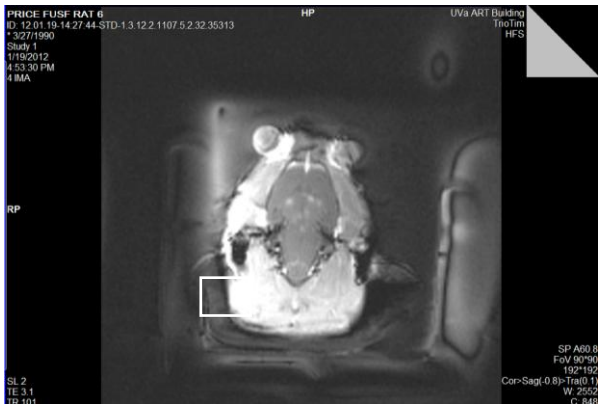

Figure 2: Spots are distinguishable when separated by 2 mm.

To validate spot-to-spot comparison within the same brain, we sonicated at the same pressure at multiple locations 2 mm from the midline and 5 mm from the surface of the skull and compared spot brightness. We determined that, for our testing conditions, contrast agent delivery to the front and rear halves

of the central hemisphere is consistent as long as care is taken to avoid obvious structural irregularities, such as the boundary between the central hemisphere and the cerebellum (Figure 3). Contrast agent delivery is also consistent within the cerebellum, but requires higher pressures than the central hemisphere.

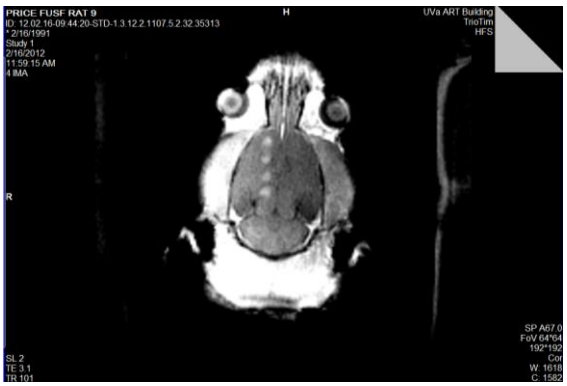

Figure 3: Demonstration of consistency in contrast agent delivery within the front and rear halves of the central hemisphere.

We are currently working on quantifying the delivery of 40 nm fluorescent nanoparticles to the central hemisphere at different pressures. Initially, it was not clear that the FUS system was capable of sonicating at different pressures under multiple target operation, but after connecting an oscilloscope to the system, we determined the setting that would allow pressure variation. We sonicated at 0.6 MPa and 0.4 MPa (interleaved to account for spatial variations in the central hemisphere, as discussed above) (Figure 4) as well as 0.5 MPa and 0.3 MPa (Figure 5). We observed that contrast agent was delivered at 0.3 MPa, but required nearly 10 minutes to appear on the MRI images, possibly indicating that different delivery mechanisms operate at very low pressures. We are now quantifying the delivery and distribution of the 40 nm particles before moving ahead with a different particle size.

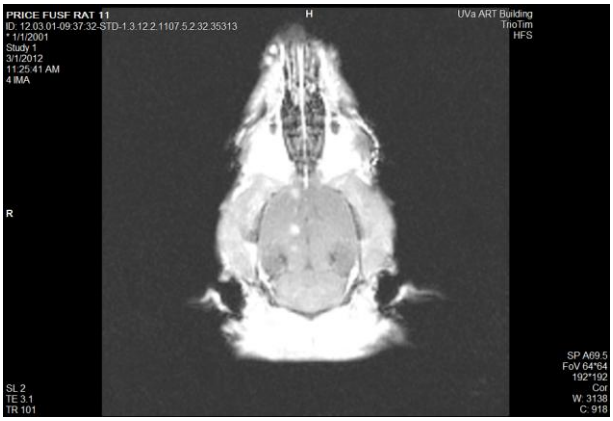

Figure 4: Interleaved sonications of 0.6 and 0.4 MPa

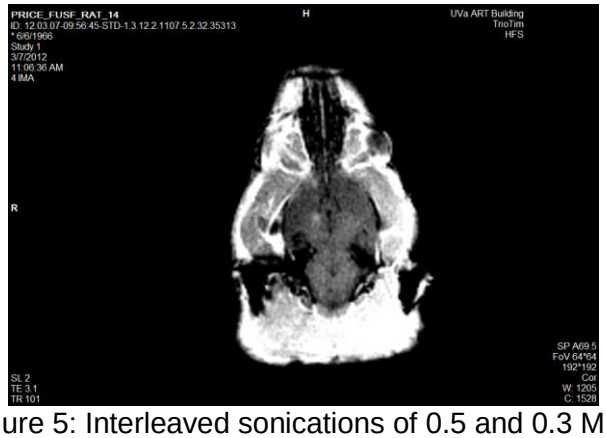

Figure 5: Interleaved sonications of 0.5 and 0.3 MPa