# Investigation on hemodynamic responses induced by peripheral and central FUS stimulation

Seongyeon Kim
*Department of Biomedical Engineering*
*Columbia University*
New York, NY, USA
sk5130@columbia.edu

Stehpen A. Lee
*Department of Biomedical Engineering*
*Columbia University*
New York, NY, USA
ssal2212@columbia.edu

Elisa E. Konofagou
*Department of Biomedical Engineering*
*Department of Radiology*
*Columbia University*
New York, NY, USA
ek2191@columbia.edu

***Abstract*—Focused ultrasound (FUS) can modulate peripheral and central nervous systems. Functional ultrasound (fUS) can monitor neural activation in the brain via neurovascular coupling. In this paper, we investigated the hemodynamic responses induced by FUS neuromodulation on the peripheral and central nervous systems in mice. As far as peripheral FUS stimulation is concerned, cerebral blood volume (CBV) increases in somatosensory region and thalamus were observed and correlates with muscle activities measured with two unipolar electrodes at tibialis anterior muscle. It is shown that nerve displacement is followed by compound muscle activation which is followed by CBV increases in two region of interests. In case of central FUS stimulation, we demonstrated highly lateralized hemodynamic responses corresponding to sonicated sides using 4-MHz FUS with displacement imaging. Based on Pearson's correlation coefficient, activated pixels showed robust hemodynamic responses over trials at different sonication sides where mean CBV increases, peaks at 3 ~ 4 s, and undergoes an undershoot before being stabilized to the baseline. In addition, the correlation map follows the spatial distribution of interframe displacement, which may indicate that the observed hemodynamic responses under our FUS parameters is mainly driven by acoustic radiation force.**

***Keywords—focused ultrasound stimulation, functional ultrasound imaging, hemodynamic response***

## I. Introduction

Focused ultrasound (FUS) has been shown capable of exciting or suppressing neurons in central nervous system and peripheral nervous system. Because of the benefits from the nature of FUS, FUS neuromodulation has been extensively studied in both nervous systems and a lot of applications utilizing neuromodulatory effects of ultrasound have been developed. As far as the peripheral nervous system is concerned, it has been found that FUS stimulation on the sciatic nerve in mice elicit physiological responses [1] and evoke mechanical sensation [2,3]. Therapeutically, FUS has been shown to be capable of reducing pain perception in subjects [4], demonstrating its possible use in the clinic for treating pain-related disease [5]. In order to measure pain perception, we can use function ultrasound (fUS). Functional ultrasound (fUS) can monitor neural activation in the brain via neurovascular coupling. It allows us to better understand how we experience sensations, especially pain, and can lead to the development of new treatments. Therefore, for the translational development of FUS neuromodulation, it is important to understand hemodynamic responses evoked by FUS stimulation in peripheral and central nervous systems. Focused ultrasound (FUS) is a noninvasive and deep-penetrating alternative that has shown promising results in animal models. Our previous finding is that FUS stimulation of the sciatic nerve leads to cerebral blood volume (CBV) increases in somatosensory cortex. However, the relationship between CBV increases, nerve displacement, and compound muscle activation induced by peripheral FUS stimulation has not been studied at length. As far as central nervous system is concerned, we previously observed not only optogenetically activated hemodynamic responses with fUS imaging [6], but also successful FUS-evoked hemodynamic responses which are widespread over cortical regions mainly due to large focal size of FUS relative to mouse brain. In this study, we investigated hemodynamic responses following FUS neuromodulation of peripheral and central nervous systems.

The objectives of this study are to demonstrate 1) hemodynamic responses induced by peripheral FUS stimulation, 2) the relationship between CBV changes, nerve displacement, and compound muscle activation, 3) central FUS-evoked hemodynamic responses corresponding to targeted and highly localized neuromodulatory effects.

## II. Methods

### A. Animal Preparation

This study was approved by the Institutional Animal Care and Use Committee of Columbia University. Four female C57BL/6 (Envigo; Indianapolis, IN, USA) ages 8 – 16 weeks were used (two animals for each nervous system). Animals were anesthetized with isoflurane (3% for induction, 2% in preparation, and 1% during fUS imaging). The mouse skull was removed for higher signal to noise ratio (SNR) in power Doppler images. Body temperature was kept at 37°C, respiratory rate was monitored, and isoflurane was modulated to achieve continuous breathing without gasping.

### B. Experimental Setup

Two different experimental setups were used in this study for fUS imaging and FUS neuromodulation of each nervous system. Peripheral FUS-fUS system consisted of a high frequency linear imaging array (MS550D, $f_c$ = 40 MHz, VisualSonics, Brea, CA, USA) for functional ultrasound imaging and a single element FUS transducer (H-108, $f_c$= 3.1 MHz, SonicConcepts, Bothell, WA, USA) which is co-aligned with 104-element phased imaging array (P12-5, $f_c$= 7.8 MHz, ATL/Philips, Bothell, WA, USA) for FUS sonication and displacement imaging. The central FUS-fUS system consisted of a 128-element linear imaging array (L22-14vXLF, $f_c$= 17.5 MHz, Vermon, USA) for functional ultrasound imaging and a single element FUS transducer (H-215, $f_c$ = 4 MHz, SonicConcepts, Bothell, WA, USA) which is co-aligned with for FUS sonication and displacement imaging. Two ultrasound research systems (Vantage 256 High Frequency Option, Verasonics, Redmond, WA, USA) were used for fUS imaging and displacement imaging, respectively, and controlled and synchronized imaging and FUS transducers.

### C. Displacement imaging for targeting of peripheral and central nervous systems

RF data was acquired from five sequential plane waves tilted from -5° to +5° and summed up to produce a compounded b-mode image at a compounded frame rate of 5 kHz. FUS was triggered before transmitting the first plane wave of third compounded frame. A GPU-accelerated delay-and-sum beamforming was used, followed by notch filtering of FUS interference [3]. Axial displacement was estimated using 1-D normalized cross-correlation [7]. Resultant nerve interframe displacement was displayed in real-time to validate placement of the FUS focus and FUS transducer was positioned using a 3D motorized positioner (Velmex, Bloomfield, NY, USA).

### D. FUS neuromodulation of peripheral and central nervous systems

In the case of peripheral FUS stimulation, the FUS transducer was placed on and coupled with a sciatic nerve before carrying out displacement imaging. Once targeting has been confirmed, sonication was carried out with FUS parameters of 1 Hz PRF, 1 ms pulse duration (PD), 5 s sonication duration (SD), and 20 ~ 24 MPa peak positive pressures. In case of central FUS stimulation, displacement imaging was also utilized for targeting either of three targets (denotes left, center, and right sonication) with a step of 1.5 mm. Amplitude-modulated pulse regime was used with FUS parameters with 1 Hz PRF, 300 ms PD, 10 s SD, and 1 ~ 4 MPa peak positive pressures.

### E. Electromyography recordings

In order to quantify muscle activities following peripheral FUS stimulation, two unipolar electrodes were inserted into tibialis anterior muscle to acquire compound muscle action potential (CMAP) form the muscle. The head was fixed in a stereotaxic frame and the legs were immobilized to reduce movement artifacts in the EMGs. A 200 ms window surrounding the FUS trigger was recorded to capture any CMAP activation. During peripheral FUS stimulation, any movement of sonicated hind limb was recorded with a camera triggered in a programmed manner simultaneously with electromyography recording.

### F. Power Doppler imaging

RF data was acquired from five sequential plane waves tilted from -7° to +7° and summed up to produce a compounded b-mode image at a compounded frame rate of 500 Hz. A GPU-accelerated delay-and-sum beamforming was used and SVD spatiotemporal filtering [8] was applied on 75 compounded frames, which generated a power Doppler image at 2 Hz.

### G. fUS data analysis

A single fUS acquisition generates a total of 180 and 270 power Doppler images depending on FUS stimulation and fUS imaging regime in peripheral and central FUS stimulation. CBV traces were normalized and subtracted by the mean CBV during baseline to calculate CBV changes. Pearson correlation coefficient was calculated with two samples; a CBV trace and a binary stimulus vector corresponding to when FUS is applied. The fUS pixels with correlation coefficient higher than 0.25 or 0.214 were considered as activated pixels depending on the number of samples in CBV traces used in analysis [9].

## III. Results and Discussion

### A. Peripheral FUS stimulation evokes CBV increases in somatosensory regions and thalamus

Peripheral FUS successfully evokes compound muscle activities in mouse hindlimb and corresponding hemodynamic response. Correlation map shows somatosensory region and thalamus are activated and exhibits CBV increases during FUS as shown in Fig. 1 (a). No activation was observed in sham (without FUS). Fig. 1 (b) displays mean CBV changes in somatosensory regions and thalamus, which showed different hemodynamic responses. FUS evokes around 5% increases in mean CBV which peaks at 2.5 s. CBV increases were shown to be higher in contralateral side compared to ipsilateral, which is similar to hemodynamic responses to somatosensory stimulation [10].

### B. CBV increases in somatosensory regions correlates with compound muscle action potential

Fig. 2 depicts a strong correlation and linear relationship between CBV increases in contralateral somatosensory region and CMAPs (p=0.0171; a two-tailed nonparametric Spearman correlation, R=0.5859). Ipsilateral side shows not significant correlation (p=0.2062, R=0.3337). Since the sciatic nerve consists of motor and sensory fibers and, under higher pressures, the more motor fibers engaged the more sensory fibers engaged under higher pressures, the result may indicate the more sensory fibers activated by FUS the higher CBV that somatosensory region exhibits.

This study was supported by the National Institutes of Health (NIH), grant number R01EB027576.

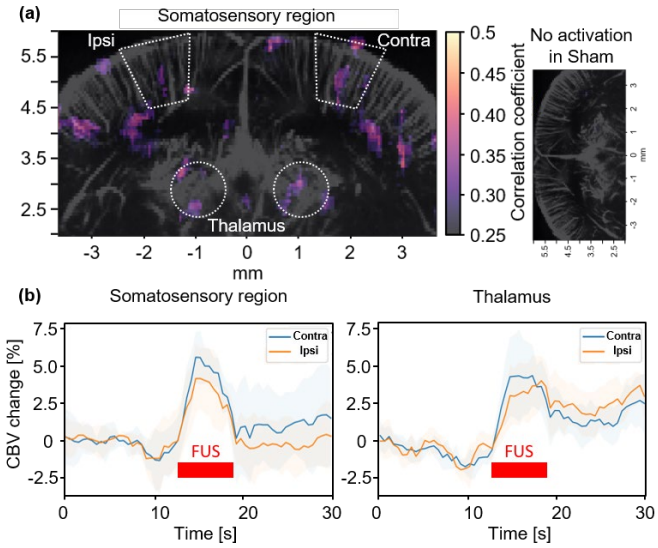


Fig. 1. Correlation map at bregma -0.5 mm and mean CBV changes in two ROIs (somatosensory region and thalamus). (a) Correlation map with segmented ROIs; somatosensory region is segmented in the white dotted trapezoid, thalamus is segmented in the dotted circle. In sham case (right), no activation was observed; no pixels with correlation coefficient more than threshold. (b) Mean CBV changes in somatosensory region (left) and thalamus (right) for 30 seconds. The red box shows when FUS is on.

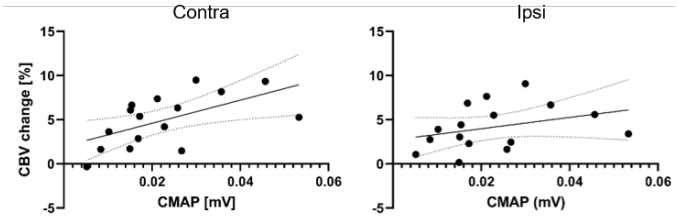


Fig. 2. Linear regression between CBV changes in somatosensory region (left: contralateral S1HL, right: ipsilateral S1HL) and CMAPs

### C. *Peripheral FUS stimulation induces nerve displacement followed by compound muscle activation followed by CBV increases.*

Fig 3 displays averaged traces of CMAPs and nerve displacement and a simple graphic to show time order between physiological events induced by peripheral FUS stimulation. Based on our observation, the nerve exhibits interframe displacement which peaks at 2 ms after when FUS is on, and it is followed by compound muscle activation, which peaks at 4 ms after and is followed by CBV increases which peaks at 2.5 s. Our hypothesis is thus as follows: once FUS displace the nerve, the activation of motor fibers in the nerve leads to signals that transmit into muscles and eventually evokes compound muscle activation. The activation of sensory fibers leads to the signals that transmit into central nervous system and eventually induces neuronal activation and corresponding hemodynamics in somatosensory regions and thalamus which are in the sensory network.

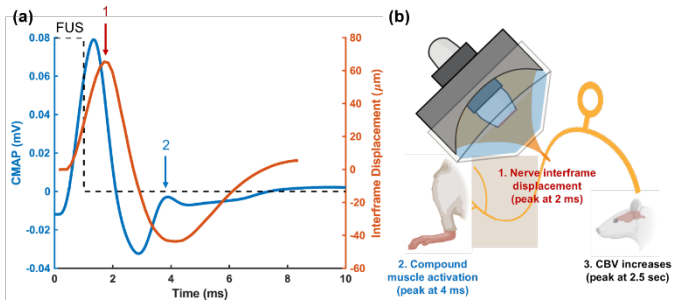


Fig. 3. (a) Averaged traces of CMAPs and nerve interframe displacement from 16 traces of 16 successful stimulations in 2 mice. (b) A graphic for time order of events after peripheral FUS stimulation.

### D. *High frequency central FUS stimulation evokes highly lateralized hemodynamic responses*

Focused ultrasound neuromodulation at 4 MHz successfully evokes hemodynamic responses. Fig. 4 displays activation map with left sonication at 1 to 4 MPa. The higher pressures are applied the more area is activated as shown the numbers on right top in the correlation maps. In addition, the successful subcortical activation induced by FUS was observed as shown in Figs. 4 and 5, which show the importance of measuring neuronal activities in deeper brain regions such as subcortical and it can be readily achieved by using fUS. Comparing correlation maps between left sonication (Fig. 4) and center sonication (Fig. 5) at 4 MPa, the observed hemodynamic responses are shown to be highly lateralized so that the pixels in only sonicated side showed activation. Fig. 5 (b) shows central FUS-evoked hemodynamic response which is an averaged CBV changes of activated pixels (n=278). FUS-evoked CBV peaks at around 3 ~ 4 s, starts decreasing, undershoots, and finally goes back to the baseline. The observed hemodynamic responses are reliable over three sonication sides and different animals.

### E. *Activation map follow the spatial distribution of interframe displacement*

Fig. 5 (c) displays an example of interframe displacement in case of targeting center area of the mouse brain. When FUS is on, the sonicated area is displaced by the FUS radiation force, which corresponds to positive interframe displacement around

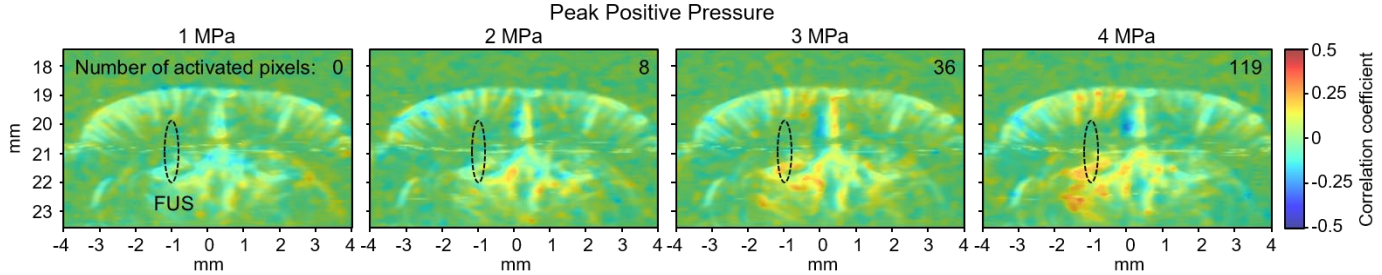


Fig. 4. Correlation map with left sonication and increasing peak positive pressures (at 1, 2, 3, 4 MPa). The number on right top denotes the number of activated pixels. The dotted area overlaid on the map shows the focus of FUS in FWHM.

the FUS focus as shown in the red around the dotted contour. In case of center sonication at 4 MPa, maximum interframe displacement in the brain was estimated to be around 2.5 μm. Interestingly, it is shown that the activation map follows the spatial distribution of interframe displacement. Comparing interframe displacement (3rd out of 59 interframes) between activated pixels and non-activated pixels, we found that interframe displacement of activated pixels is quantitatively higher than that of non-activated pixels. It may indicate that acoustic radiation force dominantly plays a role in FUS neuromodulation that we observed.

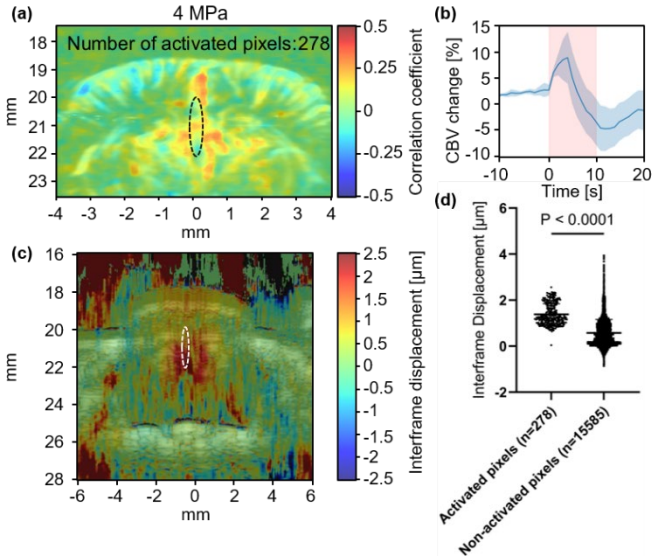


Fig. 5. (a) Correlation map with center sonication at 4 MPa. The black dotted area overlaid on the map shows the focus of FUS in FWHM. (b) Mean CBV changes of activated pixels (n=278). The shaded area denotes when FUS is on. (c) Interframe displacement at 3rd interframe out of 59 interframes. The white dotted area overlaid on shows the focus of FUS in FWHM. (d) Interframe displacement of activated pixels and non-activated pixels. Interframe displacement of activated pixels is shown to be significantly higher than that of non-activated pixels ($p<0.0001$).

## IV. Conclusion

In this study, we investigated the hemodynamic response evoked by focused ultrasound stimulation on both peripheral and central nervous system in mice. We showed that peripheral FUS stimulation evokes CBV increases in the somatosensory region and the thalamus, which correlate with compound muscle activation in hindlimb. Furthermore, we showed that nerve displacement (peaks at 2 ms after FUS is on) followed by compound muscle activation (peaks at 4 ms after), which is followed by CBV increases which peaks at 2.5s. As far as central nervous system is concerned, we demonstrate, for the first time, highly lateralized FUS-evoked hemodynamic responses in mice using high frequency FUS at 4 MHz and successful functional imaging of subcortical activation induced by focused ultrasound neuromodulation. Importantly, we adapted our displacement imaging technique to the brain and it allows us to achieve highly accurate targeting. We found that the activation map follows the spatial distribution of interframe displacement in the brain. In addition, activated area is shown to suffer higher interframe displacement than non-activated area. These results may suggest that FUS-evoked hemodynamic responses that we observed are mainly attributed to acoustic radiation force. Future work includes investigation on hemodynamic responses induced by peripheral and central FUS stimulation in 3D and an approach of utilizing acoustic lens to achieve highly targeted and localized hemodynamic responses [11].

## V. Acknowledgments

This study was supported by National Institutes of Health (R01EB027576). The authors are grateful to Pablo Abreu, MA, for his administrative assistance. The authors also thank Erica Paige Mccune, MS, Jonas Bendig, MD, Christian Aurup, PhD, Aparna Singh, PhD, and Sam Blackman, BS, for their insightful discussions.

## References

[1] Blackmore, J., Shrivastava, S., Sallet, J., Butler, C. R., & Cleveland, R. O. (2019). Ultrasound neuromodulation: a review of results, mechanisms and safety. *Ultrasound in medicine & biology*, *45*(7), 1509-1536.

[2] Downs, M. E., Lee, S. A., Yang, G., Kim, S., Wang, Q., & Konofagou, E. E. (2018). Non-invasive peripheral nerve stimulation via focused ultrasound in vivo. *Physics in Medicine & Biology*, *63*(3), 035011.

[3] Lee, S. A., Kamimura, H. A., Burgess, M. T., & Konofagou, E. E. (2020). Displacement imaging for focused ultrasound peripheral nerve neuromodulation. *IEEE transactions on medical imaging*, *39*(11), 3391-3402.

[4] Legon, W., Rowlands, A., Opitz, A., Sato, T. F., & Tyler, W. J. (2012). Pulsed ultrasound differentially stimulates somatosensory circuits in humans as indicated by EEG and FMRI. *PloS one*, *7*(12), e51177.

[5] Lee, S. A., Kamimura, H. A., & Konofagou, E. E. (2020). Displacement imaging during focused ultrasound median nerve modulation: A preliminary study in human pain sensation mitigation. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control*, *68*(3), 526-537.

[6] Aurup, C., Pouliopoulos, A. N., Kwon, N., Murillo, M. F., & Konofagou, E. E. (2023). Evaluation of Non-invasive Optogenetic Stimulation with Transcranial Functional Ultrasound Imaging. *Ultrasound in Medicine & Biology*, *49*(3), 908-917.

[7] Luo, J., & Konofagou, E. E. (2010). A fast normalized cross-correlation calculation method for motion estimation. *IEEE transactions on ultrasonics, ferroelectrics, and frequency control*, *57*(6), 1347-1357.

[8] Demené C, Deffieux T, Pernot M, et al. Spatiotemporal Clutter Filtering of Ultrafast Ultrasound Data Highly Increases Doppler and fUltrasound Sensitivity. IEEE Trans Med Imaging. 2015;34(11):2271-2285. doi:10.1109/TMI.2015.2428634

[9] Macé, E., Montaldo, G., Cohen, I., Baulac, M., Fink, M., & Tanter, M. (2011). Functional ultrasound imaging of the brain. *Nature methods*, *8*(8), 662-664.

[10] Jung, W. B., Im, G. H., Jiang, H., & Kim, S. G. (2021). Early fMRI responses to somatosensory and optogenetic stimulation reflect neural information flow. *Proceedings of the National Academy of Sciences*, *118*(11), e2023265118.

[11] Jiménez-Gambín, S., Jiménez, N., Pouliopoulos, A. N., Benlloch, J. M., Konofagou, E. E., & Camarena, F. (2021). Acoustic holograms for bilateral blood-brain barrier opening in a mouse model. *IEEE Transactions on Biomedical Engineering*, *69*(4), 1359-1368.