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***Abstract* — Functional ultrasound (fUS) is a relatively new imaging modality to study the brain with a high spatiotemporal resolution and a wide field-of-view. In fUS detailed images of cerebral blood flow and volume are used to derive functional information, as changes in local flow and/or volume may reflect neuronal activation through neurovascular coupling. Most fUS studies so far have been performed in rats. Translating fUS to mice, which is a favorable animal model for neuroscience, pleads for a higher spatial resolution than what has been reported so far. As a consequence the temporal sampling of the blood flow should also be increased in order to adequately capture the wide range in blood velocities, as the Doppler shifts are inversely proportional to the spatial resolution. Here we present our first detailed images of the mouse brain vasculature at high spatiotemporal resolution. In addition we show some early experimental work on tracking brain activity upon local electrical stimulation.**

***Index Terms*—fUS, mice brain, high frequency, µDoppler**

I. Introduction

The increasingly popular technique of high frame rate ultrasound imaging, which involves unfocussed transmissions, parallel detection and post-processing focusing has enabled a new neuroimaging tool called functional Ultrasound (fUS) [1]. In fUS, detailed images of cerebral blood dynamics are captured by visualizing the minute motions induced by moving blood cells and possibly other vascular related motion such as dilation and vasoconstriction. Changes in the dynamics of the cerebral blood supply to specific locations may reflect local changes of metabolic activity upon neuronal activation. This process is referred to as neurovascular coupling (NVC) and forms the foundation of many neuroimaging techniques such as PET and fMRI [2]. The 'functional' information brought by these imaging techniques is often obtained by correlating a known stimulus pattern with every pixel over time or by seeking a shared response across multiple pixels by e.g. inter-pixel correlations or more advanced statistical approaches such as Support Vector Machines or Graph Signal Processing [3].

Most fUS work so far has been performed in relatively large rodents such as rats [1,4] and ferrets [5], and even in pigeons [6]. Translating fUS to mice, which is a far more interesting animal for neuroscience, involves some technical advancements. Not only in terms of scaling up the frequency of the ultrasound in order to recover the smaller vasculature of the mouse brain, but also potentially scaling up the temporal frequency as the Doppler shifts are inversely proportional to the spatial resolution and the faster vascular dynamics involved with the smaller mouse brain as compared to rats.

To this end we have established high frequency fUS in our lab to study the mouse brain at sufficient spatial (50 µm at a 30 MHz ultrasound frequency) and temporal (0.1 ms at a 10 kHz framerate) resolution. Below we discuss the details of our high frequency fUS implementation geared towards mouse brain imaging and show some compelling examples of an experiment where we electrically stimulate the brain and track the activation pattern over time.

II. Materials And Methods

A. Animal model and Surgical Procedure

A total of n=3 adult anesthetized C57BL/6 mice (Charles River, NL) were subjected to either a whisker stimulation or an electrical cerebellar stimulation paradigm (see section C and D). After induction anesthesia (5% isoflurane in O_2), a large craniotomy in combination with a titanium pedestal placement was performed exposing the bilateral barrel fields or bilateral cerebellum plus motor cortex respectively.

After bone removal, the exposed brain was covered using an acoustically transparent and sterilized 150 µm thick film of Polymethylpentene (TPX™) material [7] placed epidurally, further stabilized by Charisma® composite, thus creating a sealed acoustic window. This procedure allows for chronic fUS studies in mice (Fig. 1).

After pedestal placement and craniotomy, mice were head-fixed in the set-up for imaging. During imaging, anesthesia was lowered (around 1% isoflurane in O_2). Throughout the experiment, body temperature was maintained at 37.6°C

B. Ultrasound acquisition and image formation

For the ultrasound data acquisition, we used an ultrasound research system (Vantage 64-LE, Verasonics Redmond, WA, USA) interfaced with a 128 element, high frequency linear array (L35-16v, 69 µm pitch) driven with a 3 cycle burst at 30 MHz. Because of the 64 channel acquisition, two transmit-receive events were needed to address the complete aperture. Because the ultrasound frequency bandwidth exceeds the Nyquist rate of the Verasonics system (31.25 MHz) we used the 67% bandwidth sampling approach as described in [8].

We developed our own CPU/GPU data processing software, to achieve continuous, real-time imaging for a long period of

time, including the storage of raw beamformed images. The major processing steps include Fourier domain beamforming [9], SVD clutter filtering and Power Doppler image (PDI) formation [10], real-time display and data storage to a 2 TB PCIe SSD. Naturally the maximum achievable temporal resolution depends on the specific imaging settings, such as how many plane wave angles are used for compounding and the duration of the pulse-echo travel time. In our implementation this temporal resolution can be as high as 0.5 ms, while maintaining real-time display (20 Hz) and continuous raw frame storage (2 KHz), when using for example 6 compounded angled plane waves and an 8.8 by 8 mm field-of-view. Note that in this case the actual plane wave transmit and receive frequency is 24 kHz. However we can increase this temporal resolution at the expense of compounding and probe heating.

For display purposes we enhanced the vascularity by high pass filtering the PDI, up-sampling by linear interpolation, gamma correction and saturation of the highest values.

C. *Whisker stimulation paradigm*

Mice were exposed to a standard air-puff whisker stimulation paradigm (for example see ref. [1] and [11]) to ensure our fUS implementation could detect functional signals associated with an external stimulus in already well-established functional areas, in this case the barrel fields.

D. *Electrical stimulation paradigm*

To further test our fUS implementation we performed an experiment where we electrically stimulated around the region of the superior cerebellar peduncle (SCP), although no histology was performed to confirm this placement. The bipolar stimulation electrode (AlphaOmega, bipolar, 0.5M, 100µm tip separation) was placed using visual guided µDoppler imaging. During each session, the SCP was stimulated with 5 periods (Fig. 5, grey bars) of 1 sec, 200Hz, 100µA biphasic pulse trains (1 ms pulse width).

III. Results

Our fUS implementation allowed for successful imaging experiments on different mice. In Fig. 1. we visualize the vasculature of a mouse brain through the acoustically transparent plastic window. Both are coronal views of the same brain at week 2 and 4 after surgery. These images are obtained by averaging 25 PDI's, which are made using 15 compounded angled transmissions and an ensemble length of 150 frames.

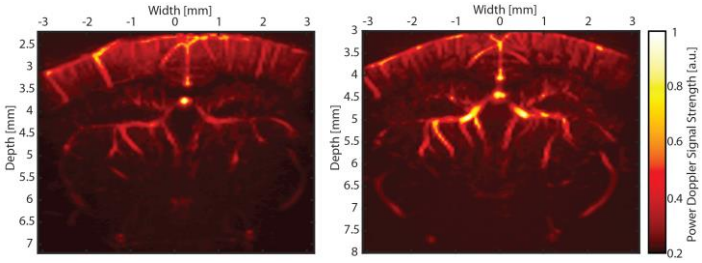

Figure 1: The mouse brain vasculature imaged through an acoustic transparent window at week 2 (left) and week 4 (right) after placement.

In Fig. 2 and 3 we show two orthogonal views of a mouse brain to emphasize the rich and detailed vasculature as seen with our high frequency fUS implementation. Figure 2 shows a coronal view -1.8 mm from bregma and Fig. 3 shows a sagittal view along the midline of the same mouse brain. Apart from the rich vasculature it also shows that the signal-to-noise ratio decreases after several millimeter. These PDI's (Fig. 2 & 3) are obtained by averaging 25 PDI's, consisting of 20 compounded angled transmissions and an ensemble length of 200 frames.

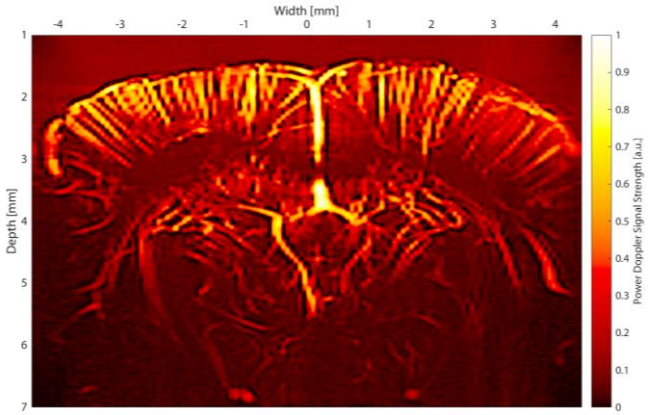

Figure 2: Coronal view of a mouse brain around bregma -1.8 mm. Cerebral cortex (among which primary sensory cortex and perirhinal cortex), habenula, hippocampus, thalamus (among which ventrobasal complex and mediodorsal nucleus), and hypothalamus. A few pronounced vessels can be identified. On the lateral ventral part of the thalamus, the ventral thalamic artery (most pronounced on the left side) can be seen ascending along the lateral border. Further laterally and dorsally than this, the anterior choroidal artery can be seen branching into the dorsal thalamic arteries.

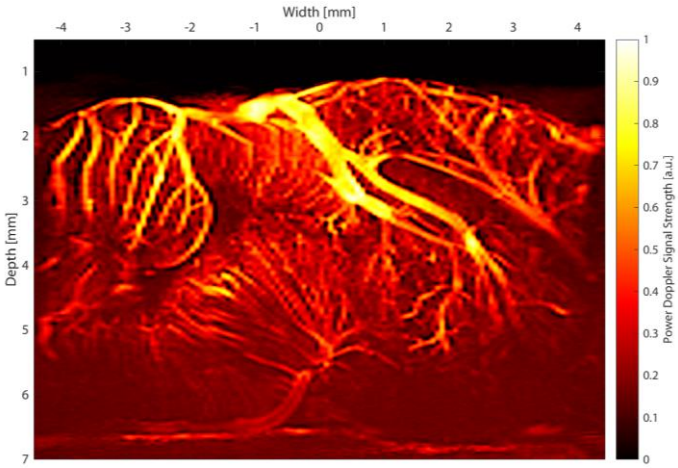

Figure 3: Sagittal view of a mouse brain along the midline. The amount of detail and wide field-of-view allows us to identify that within the cerebellum, the penetrating arterioles resemble the lobular structure found in the histological architecture of the cerebellum. Over the superior colliculus, the transverse sinus can be seen. From that point, a large vein (great cerebral vein of Galen, GCVoG) can be seen descending together with the longitudinal hippocampal vein in between the retrosplenial granular cortex and the superior colliculus. Deeper in the brain, one vessel can be seen running over the corpus callosum (presumably azygos pericallosal artery), one over the rostral border of the thalamus, the thalamostriate vein (extension of GCVoG) and one branch penetrating into the thalamus (extension of the longitudinal hippocampal vein). To conclude, from the ventral side of the brain, the basilar artery can be seen ascending into the interpeduncular fossa, from which point (posterior cerebral artery) numerous small branches can be observed into caudal, dorsal and rostral direction, covering the majority of the brainstem at this level.

The above examples provide proof that our fUS implementation is capable of visualizing the mouse brain vasculature with sufficient detail. Now we focus on extracting functional information from these images by employing a simple whisker paradigm. The whiskers pathways to the barrel cortex have been well studied and form a reliable model for e.g. investigating sensorimotor integration or neural development [11]. For this initial test we adopt the implementation as presented by Macé et al [1]. Figure 4 shows the PDI (grayscale) with in color overlay the functional correlation map. Only correlation values higher than 0.3 are shown. Increased activity can be observed in both the barrel field and thalamus.

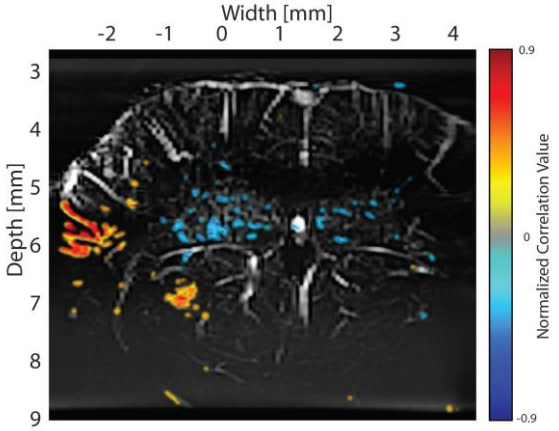

Figure 4: A whisker stimulation experiment as a paradigm for fUS. The left panel depicts the activation map acquired using fUS in a whisker stimulation experiment, showing that the left barrel field and the ventrobasal complex of the thalamus are involved in the task.

In Fig. 5. we show a few PDI's of a mouse brain vasculature in sagittal view with in color overlaid the average correlation with the stimulus signal at several time lags. The stimulus signal was applied using a bipolar stimulation electrode that was inserted below the cerebellum, presumably in close proximity of the SCP.

IV. Discussion and Conclusions

In this paper we have presented our high frequency implementation of fUS tailored towards whole mice brain imaging for the neurosciences allowing for a spatial resolution of 50 µm and a temporal resolution lower than 0.1 ms. We have demonstrated the use of high frequency fUS through an acoustically transparent plastic window, to visualize in high detail the mouse brain vasculature, while being capable of detecting vascular responses upon sensory and electrical stimulation. The observed vascular responses are anatomically conform previous electrical experiments where the whiskers are known to activate the ventrobasal complex in the thalamus and the barrel cortex, and the SCP is known to activate the thalamus, (indirectly) cerebral cortex, and possibly the cerebellar cortex via backpropagation and subsequent nucleocortical connections.

These are examples of well-known functional networks that, when stimulated, are adequately measured with use of high frequency fUS. This provides the opportunity to investigate less well-known networks and structures with fUS on a large-scale

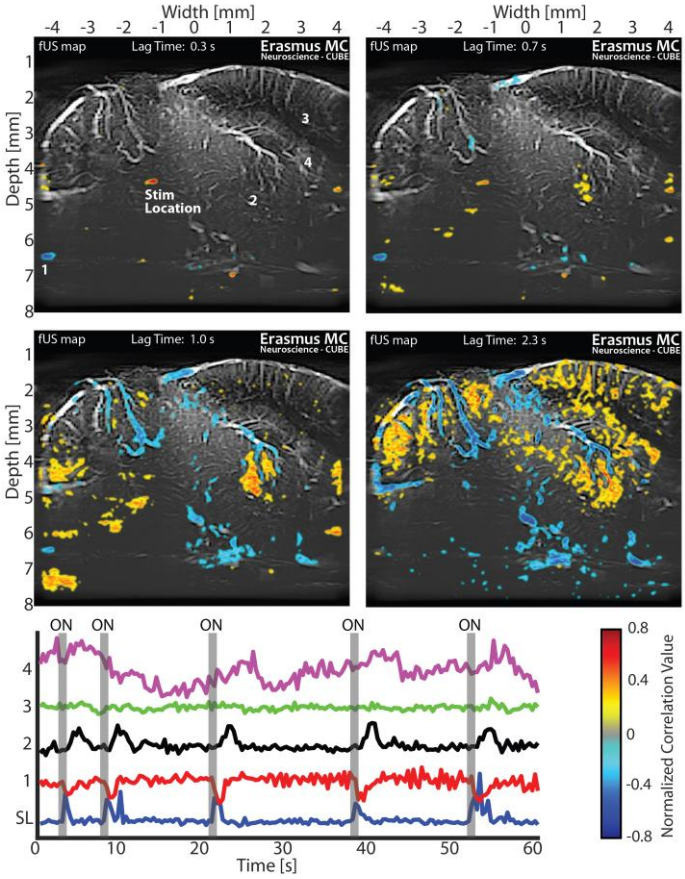

Figure 5: Activation patterns visualized with fUS upon electrical stimulation. The four sagittal oriented PDI's with in color overlaid the correlation with the stimulus pattern show the progression of the activation over time. Only correlation values larger than 0.2 are shown. Bottom plot depicts the 5 signals from 5 different regions in the brain. The blue bottom signal, Stim Location (SL), is the location of the stimulation electrode.

while maintaining high spatial detail of the vasculature due to our high frequency implementation.

We also experienced that reducing motion artefacts to a minimum allowed for a very stable baseline signal and detailed images at greater depths. This reduction was attained by the use of the pedestal directly attached the rostral part of the skull and with the use of an actively air damped experimental table. The probe cable was actively kept loose such that vibrations from outside the table could not propagate well along the transducer cable.

By showing that fUS can be used to track locally induced activation upon electronic stimulation, as we did, or potentially other forms of stimulation such as through optogenetics we again reaffirm the unique capabilities of fUS as a new and powerful technique for neuroscience. However, to optimally utilize fUS for this type of connectivity studies, many aspects still have to improve, among which detailed studies on the neuronal activity underlying the fUS signal and the precise biophysiological process(es) underlying the fUS signal.

Our future work will now further focus on unraveling brain dynamics during learning tasks in the cerebellum as well as technical advancement of the fUS imaging tool and the computational embedding of this technique.

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