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Fully-automatic ultrasound-based neuro-navigation : The functional ultrasound brain GPS

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ABSTRACT

Recent advances in ultrasound imaging triggered by ultrafast plane waves transmission have rendered functional ultrasound (fUS) imaging a valuable neuroimaging modality capable of mapping cerebral vascular networks, but also to indirectly capture neuronal activity with high sensitivity thanks to the neurovascular coupling. However, the expansion of fUS imaging is still limited by the difficulty to identify cerebral structures during experiments based solely on the Doppler images and the shape of the vessels. In order to tackle this challenge, this study introduces the vascular brain positioning system (BPS), a GPS of the brain. The BPS is a whole-brain neuro-navigation system based on the on-the-fly automatic alignment of ultrafast ultrasensitive transcranial Power Doppler volumic images to common templates such as the Allen mouse brain Common Coordinates Framework. This method relies on the online registration of the complex cerebral vascular fingerprint of the studied animal to a pre-aligned reference vascular atlas, thus allowing rapid matching and identification of brain structures. We quantified the accuracy of the automatic registration using super-resolution vascular images obtained at the microscopic scale using Ultrasound Localization Microscopy and found a positioning error of 44 μm and 96 μm for intra-animal and inter-animals vascular registration, respectively. The proposed BPS approach outperforms the manual vascular landmarks recognition performed by expert neuroscientists (inter-annotator errors of 215 μm and 259 μm). Using the online BPS approach coupled with the Allen Atlas, we demonstrated the capability of the system to position itself automatically over chosen anatomical structures and to obtain corresponding functional activation maps even in complex oblique planes. Finally, we show that the system can be used to acquire and estimate functional connectivity matrices automatically. The proposed functional ultrasound on the fly neuro-navigation approach allows automatic brain navigation and could become a key asset to ensure standardized experiments and protocols for non-expert and expert researchers.

Introduction

In the recent years, the advent of ultrafast ultrasound imaging at thousands of frames per second has allowed ultrasound to gain two orders of magnitude of sensitivity to small blood flow [1,2]. This new capability to image small cerebral vessels allowed ultrasound to enter the field of neuroimaging using cerebral blood volume to measure indirectly brain activation through the neurovascular coupling. Functional Ultrasound (fUS) can provide functional information, such as activation maps or functional connectivity[3] in the brain, with high spatial and temporal resolution. In preclinical imaging, a large number of studies have been published from small to large animal models demonstrating its potential in neuroscience and clinical research[4]. These studies have been carried out mainly with fUS neuroimaging scanners driving linear ultrasonic probes. Such approach allows high sensitivity acquisition and to image directly through the skull without complex animal preparation in mice, the most used animal model in neuroscience research. Several approaches have been proposed to tackle the challenging whole brain 3D imaging in fUS either based on moving linear arrays, matrix arrays or RCA arrays. However, both matrix arrays and RCA arrays have a limited sensitivity which is not sufficient to perform transcranial imaging even in mice and require invasive surgery. Thus, high sensitivity linear probes remain to date the best suited technology for non invasive fUS imaging. However, positioning the linear array over the correct slice to image remains challenging. Unlike MRI, ultrasound imaging provides poor anatomical images especially through the skull but provides excellent mapping of the vascular anatomy [5]. Although experts can learn after a long training curve to navigate through vascular images and position the array correctly with respect to functional brain areas, it remains a crucial need for a robust and automated approach to lower this know-how barrier, as well as to improve standardization and reproducibility between different operators and different animals or experiments.

In this paper, we investigated how the Doppler contrast producing a vascular fingerprint, can be used 'on the fly' to provide accurate neuronavigation capability within functional ultrasound experiments.

Brain positioning principle

A classical functional ultrasound imaging experiment starts by positioning the ultrasonic probe over the targeted functional regions[6,7]. For this, users take advantage of the high sensitivity of ultrafast Doppler imaging to reveal and identify vascular landmarks such as vessel shape or branching within the mouse brain. Using a motorized system, they move the probe over different brain areas and image several canonical planes until they recognize those vascular

clues, usually restricting themselves to coronal slices which are much easier to apprehend (Fig. 1A). They can then perform functional ultrasound acquisition with high frame rate either on a single slice or on several slices [8,9]. fUS imaging remains thus inherently limited by the expertise required to neuro-navigate oneself within the brain prior to acquisition. The brain positioning system (BPS) proposed here provides a fully-automatic approach to solve this problem during the experiment and provides a familiar anatomical context and automatic probe positioning in any desired plane and orientation for the whole mouse brain (Fig. 1B). Our approach relies on the coupling of an online automatic registration (Fig. 2A) of a 3D Doppler volume - acquired in the very beginning of the imaging session - to a 3D Doppler reference, which was pre-aligned once with any modality of interest (Fig. 2B). This modality can for instance provide anatomical or functional information and we illustrate mainly in this work the use of the Allen Brain Mouse atlas [10] to search, display and finally reach - thanks to the coupling with a motorized system - specific structures to image (Fig. 2C).

Many studies have explored the possibilities of using vascular information to register volumes either intra or inter modalities. In clinical neuroscience, an active strong area of research has been to explore brain shift correction during intraoperative surgery in order to correct brain MR images from live Doppler data [11]. By leveraging the brain vascular footprints and integrating the approach directly in the functional ultrasound experimental workflow, we can improve the quality and standardization of the experimental data. In that regard, our 'on the fly' BPS approach brings functional ultrasound imaging closer to fMRI, where MR images can be used to position acquisition slices and MR contrast templates can be used by software suites such as SPM or FSL to warp and normalize the datasets to a common space for group-level analysis [12,13].

We first illustrate the specificities and invariants of the registration of those vascular footprints obtained from 3D Doppler volumes by evaluating intra-animal and inter-animal pairs of image and resulting cross-correlation profiles. We then compare the automatic BPS registration results with landmarks-based manual registration positioned by trained experts with more than 40 cumulative years in neuroscience and neuroanatomy. Finally, in order to evaluate the accuracy of the Doppler registration, we use BPS-aligned super-resolution angiographic images with tens of micrometers spatial resolution to evaluate positioning errors with tens of micrometers accuracy.

The BPS is then evaluated for functional activation mapping in complex, oblique planes, and for functional connectivity assessment with the added benefit of automatic regions of interest extraction.

Material and methods

Methods were carried out in accordance with relevant guidelines and regulations and in compliance with ARRIVE guidelines.

Animals

All animals received humane care in compliance with the European Communities Council Directive of 2010 (2010/63/EU) and the study was approved by the institutional and regional committees for animal care: CEEA (Comité d'Ethique pour l'Expérimentation Animale) numéro 59 - "Paris Centre et Sud" Protocole # 2017-23). Twenty-four adult mice (male C57BL/6 Rj, 8-14 weeks old, from Janvier Labs, France) were used for this study. At arrival, they were housed 4 per cage with a 12h light/dark cycle, constant temperature at 22°C and food and water ad libitum. Before the beginning of the experiments, animals were given a 1-week minimum acclimatization period to housing conditions.

Anesthesia and animal preparation

Animals were anesthetized by intraperitoneal injections of a bolus of Ketamine 60mg/kg and Medetomidine 1mg/kg. Once anesthetized, animals were placed in a stereotaxic frame (David Kopf, Tujunga, USA). After shaving the hair on the head using a depilatory cream (Clarins, France), echographic gel was applied and the ultrasound probe was then placed in contact with the gel. Imaging was therefore performed through the skin and skull. Anesthesia was maintained by subcutaneous infusion of Ketamine 20mg/kg/h, Medetomidine 0.3mg/kg/h using a push seringue (KD Scientific).

Ultrafast Ultrasound Power Doppler imaging sequence: Transcranial ultrafast acquisitions were performed using the Iconeus One scanner (Iconeus, Paris, France) driving a 15-20 MHz probe (128 elements, 0.11mm pitch) mounted on a motorized setup. Each image at a unique position of the probe was obtained from 200 compounded frames acquired at 500 Hz frame rate. Each of these raw ultrasonic frames was built using 11 tilted plane waves (-10°, -8°, -6°, -4°, -2°, 0°, 2°, 4°, 6°, 8°, 10°) acquired at 5500 Hz pulse repetition frequency. Power Doppler images were obtained after blocks of 200 compounded frames were processed using a spatiotemporal Singular Values Decomposition (SVD) clutter filter[14] to discriminate blood flow from tissue motion signal. The imaging device enables 100 μm \times 100 μm in plane resolution with a slice thickness of approximately 400 μm . Successive 2D slices were acquired with 0.2 mm spatial steps to reconstruct 3D vasculature with (100 x 100 x 400) μm^3 resolution. Functional ultrasound imaging sessions were performed by real-time continuous acquisitions of successive blocks of 400 ms (200 compounded frames at 500 Hz) resulting in a 2.5 Hz Doppler frame rate.

Ultrafast Ultrasound Localization imaging : Initial suspension of microbubbles solution was obtained by dissolving 2.5 mg powder in 5 mL NaCl solution. 70 μL of the initial solution was injected through the catheter placed in the tail vein of the mouse. Iconeus One scanner was used to acquire 600 blocks of 400 compounded frames acquired with 9 angles (-8°, -6°, -4°, -2°, 0°, 2°, 4°, 6°, 8°) at 1000 Hz frame rate. Microbubbles signal is separated from the surrounding tissue signal using a SVD filter. Microbubbles are tracked using an algorithm based

on the Hungarian method[15]. Each track is smoothed with sliding average, interpolated and projected on a 2D grid to reconstruct the density and velocity maps of the microbubbles[16,17].

Reference Doppler and brain template offline alignments: A 3D Doppler volume was first acquired using the ultrafast Doppler tomographic approach[5] using the same parameters as described in the Power Doppler section with 19 rotations. Briefly, high sensitive Doppler images of the mouse brain were acquired at different positions and angles and used to reconstruct an isotropic high resolution of 3D vascular network. This reference Doppler was then semi-automatically aligned to the Allen atlas two-photon template using the elastix module of the slicer3D software[18,19,20].

On the fly vascular registration and neuronavigation: During the experiments, a 3D Doppler volume was acquired and then automatically registered using a dedicated prototype software.

First, Doppler data was used to automatically perform an affine monomodal registration with the reference Doppler. The chosen registration algorithm is based on Mattes mutual information metric maximization[21,22] to measure how similar the images are and an evolutionary optimizer[23] to find at each iteration a set of parameters that produce the best registration result (Mathworks, Natick, Massachusetts, USA). To ensure the reproducibility of registration, all the spatial samples were used to compute the probability density over 50 bins enabling mutual information estimation between the two data sets. The software then automatically applied the geometric transformations from the Allen Atlas space to the 3D volume through the Reference scan to overlay the Allen structures directly on the Doppler scan and to successively acquired scans. For neuronavigation and automatic positioning of the probe, the software is used to define a new virtual imaging plane from the position of two markers that can be set by the user on top of the overlaid brain structures. The motor positions of the robotic platform are then computed automatically from the plane coordinates with an inverse kinematic solver (*inverseKinematics*, Mathworks) and are used to move the probe over the desired imaging plane.

Registration accuracy estimation from Power Doppler images

3D linear scans were acquired with $n=5$ C57BL/6 mice for Power Doppler based registration estimation. Each scan has a 6 mm antero-posterior range covering the entire Bregma-Lambda region with $(100 \times 100 \times 400) \mu\text{m}^3$ resolution.

Normalized Cross-correlation

To illustrate the similarity between the registered volumes we computed the 3D normalized cross-correlation of the Doppler volumes. We then measure the peak value, peak location and

peak width as simple metrics of volumes similarity, shift and spatial precision. Please note that the correlation is not used for the registration but only for illustration.

Registration accuracy estimation from the comparison of the BPS with manually positioned vascular landmarks

Definition of vascular landmarks

A common set of vascular landmarks were chosen by two experts in the field and can be seen in Supplementary figure 1.

The first vascular landmark VL1 is located at the junction between right and left aspects of the medial choroid plexus (yellow arrow). The plane can be recognized by the circular shape of the choroid plexus (arrow 1) and artery in the ventral aspect of the brain (arrow 2).

The second vascular landmark VL2 is the change of direction in the AchA (yellow arrow), from angle to vertical. The plane is recognizable by the presence of the AchA [24] (Anterior Choroidal Artery, arrow 1) and the internal carotid (arrow 2).

The third vascular landmark VL3 is located on the right ending of the medial pointy part of the choroid plexus (yellow arrow). The plane is recognizable by the round and descending aspect of the thalamic artery (arrow 1) and presence of the AchA (arrow 2).

The fourth vascular landmark VL4 is the medial and most ventral joining point of PCA (Posterior cerebral artery) medially (yellow arrow). The plane is recognizable by the PCA. Note that 1) the planes containing the markers 3 and 4 are very close and sometimes the same plane of imaging, and 2) the marker 4 is not the joining point a few millimeters more dorsally (arrow 2).

Registration and Resampling

We first considered a group of 5 acquisitions (3D Power Doppler Doppler with 100 x 100 x 400 μm^3 resolution; 3 days between the first and the last acquisition) from the same animal to evaluate longitudinal intra-animal registration. Successively, each of the acquisitions was used alternatively as reference volume to register the other acquisitions resulting in 20 registration operations. Moved volumes were obtained after registration and resampling within reference space.

Manual annotation of registered volumes using the chosen vascular landmarks

For each pair of acquisitions (reference and moved volumes), four vascular landmarks are manually labeled within each volume by two experts. For each landmark a pair of coordinates is then recorded in the reference space.

Discrepancies between BPS estimation and neuroanatomists manual annotations

Since moved volume is resampled within reference space, for each landmark any difference between the two values estimates the shift between BPS automatic registration prediction and the experts annotation. These discrepancies are computed as 3D distance shifts and averaged over all the pairs of registration operations.

Discrepancies between neuroanatomists manual annotations

Since the two experts manual annotations were performed in the same conditions, raw differences between recorded coordinates for both experts allows to estimate any discrepancies between the manual annotations. These discrepancies are computed as 3D distance shifts and averaged over all the registration operations.

For inter-animal assessment, the same method was performed with a second group including 5 acquisitions from 5 different mice.

Registration accuracy estimation with ULM super resolution images

Finer accuracy measurements of the vascular registration were performed using super resolution images of the vascular structures thanks to ULM [16]. The BPS was used to position the probe and perform a first Doppler and ULM acquisition in specific slices. In a second, independent experiment, the BPS was used again to reposition the ultrasonic probe on the very same imaging plane and both new Power Doppler and ULM images were acquired. The reference volume for the BPS could be either from the same animal (direct registration) or from another previous animal (indirect registration). The direct registration was intended to evaluate the vascular to vascular registration accuracy with a same animal as used in a longitudinal study while the indirect registration evaluates the BPS accuracy with a pre-aligned template acquired from a different animal. The super resolved images from the first and second experiments were then compared to measure the misalignment (as 2D translations) due to the BPS in both coronal and sagittal planes. For each pair of super resolution images a displacement map is computed in both lateral and axial directions (Supplementary Fig. 2) using non parametric non-rigid [25,26] registration. The displacement is averaged over the entire vasculature to provide single misalignment value for each direction from this pair of acquisitions. This operation was repeated for 15 pairs of coronal acquisitions (X,Z directions) and 7 pairs of sagittal acquisitions (Y, Z directions) performed in $n=3$ C57BL/6 mice for direct registration group. The indirect registration group included 20 pairs of coronal acquisitions and 10 pairs of sagittal acquisitions performed in $n=6$ C57BL/6 mice.

Task-evoked functional imaging: A first marker was set in the center of the left primary somatosensory, barrel field area and a second marker in the center of the right primary visual

area (Fig. 6, red box). This allows automatic probe positioning on an oblique imaging plane which encompasses both functional areas targeted. Then we successively performed whiskers stimulation and visual stimulation. A few posterior and caudal right whiskers were stimulated with the following pattern: 30 s baseline followed by three consecutive trials of 30 s ON and 30 s OFF for a total acquisition duration of 210 s. Red LED was placed at 3cm in front of the left eye to perform visual stimulation with the same pattern as for whiskers.

Activation maps were obtained by computing Z-scores using a generalized linear model analysis (GLM) between each voxel temporal signal and the stimulus pattern. Thus multiple hypotheses are tested to compare the entire imaging area and a conservative Bonferroni correction is applied by dividing the overall conventional p-value (0.05) by the number of independent hypotheses (number of voxels) to compensate for the increase of type I error. Therefore the individual voxel activation is tested with p-value < 0.00001 level of significance. Z-scores maps are overlaid on the baseline Power Doppler image. Anatomical regions from the Allen Mouse Brain Common Coordinate Framework are automatically positioned and displayed for reference by the BPS.

Functional connectivity analysis:

Under stable conditions, three acquisitions of 600 s at different slices were performed for resting state connectivity analysis. A low-pass filter with 0.1 Hz cutoff frequency was used to select spontaneous low-frequency CBV fluctuations. We used the BPS to automatically register the acquisitions to the Allen brain atlas. This enables the identification and automatic extraction of about 70 regions of interest based on the Allen ontology grouped in larger regions (Isocortex , Thalamus and Hypothalamus). The connectivity matrix is obtained by computing the normalized Pearson correlation between spatial averaged and temporal filtered signals extracted from each selected ROI. The correlation coefficients are color-coded for representation. For seed-based analysis, several seeds identified from the automatic registration on Allen Brain Atlas were selected. Seed-based correlation maps were formed by computing the normalized Pearson correlation coefficient between the average signal of the seeds and each individual voxel of the data. The correlation coefficients were color-coded and overlaid on the baseline Power Doppler image. Anatomical regions from the Allen Mouse Brain Common Coordinate Framework are displayed including the seed region (with different color).

Results

Automatic registration is as accurate as neuroanatomist expert annotation

The accuracy of the vascular registration is a critical point in the proposed neuro-navigation approach. We first assessed the accuracy of the ultrafast doppler vascular to vascular volume registration in the same mouse at different time points. Inherent spatial shifts between acquisitions is inevitable during the experiment and the mouse installation in the stereotactic frame. The green-magenta representation illustrates this initial misalignment between the two datasets before registration (Fig. 3A). The first acquisition (time point t0) is displayed in green) while the second acquisition from the same animal acquired at time point t1 (24h after) is considered to be the moving volume (magenta) that we want to register. After registration and resampling of the second volume to the first one, a good match is found by the registration algorithm between the two vascular datasets (Fig. 3A) as illustrated by the dominance of white color. On the same animal, the normalized cross-correlation between the reference and registered volumes shows a strong (value = 0.9) and sharp (width at half-maximum $dx = 0.65$ mm, $dy = 1.34$ mm, $dz = 0.64$ mm) peak located in the center (Fig. 3C). As reference, autocorrelation (same volume) yields a full width at half maximum ($dx = 0.5$ mm, $dy = 1.15$ mm, $dz = 0.55$ mm).

For registration between different animals and although the details from vascular networks are different, the general vascular architecture still enables a good registration mainly thanks to large vessels (Fig. 3B) as illustrated by the white areas located in larger vessels in the green-magenta representation. The normalized cross-correlation between the reference and registered volumes demonstrates a lower correlation (value = 0.64) and a wider ($dx = 1.07$ mm, $dy = 1.71$ mm, $dz = 0.83$ mm) peak which is expected because the vascular fingerprints are now different and the inherent uncertainty higher.

We then evaluated the accuracy of the registration with vascular landmarks. First the result of registration and resampling automatically performed is illustrated in Figure 4. Automatic predictions of landmarks placed in the reference (green point Fig. 4A) are illustrated in red in moved volumes for both intra-animal (Fig. 4B-C) and inter-animal registration (Fig. 4D-E). We can note a good reproducibility of registration of these landmarks both between acquisitions in the same animal and between animals.

Then we compared the discrepancies of the landmarks placed by trained experts in different datasets with those automatically predicted by the registration as described in Material and methods. The results are reported in Table 1. First, we compared independently each expert annotation to the BPS registration. Automatic registration had an average shift of 120 ± 84 μ m (ranging from 78 ± 58 to 158 ± 94 μ m) for the four vascular landmarks compared to the first neuroanatomist expert annotation. The second expert annotated the landmarks with an average 130 ± 82 μ m (ranging from 105 ± 70 to 155 ± 90 μ m) shift compared to the automatic registration. As a comparison, we compared the two experts' annotations to one another. Their estimations were globally misaligned by 215 ± 87 μ m (ranging from 184 ± 94 to 253 ± 101 μ m). Thus, we found that for any of the four landmarks and for the overall annotation the discrepancy between experts annotations was higher than the one between each individual expert

annotations and the BPS prediction. It shows that the accuracy of the automatic registration is well within the expert manual positioning error. Notably, experts needed about 5 minutes to navigate the slices and annotate the four landmarks within a pair of acquisitions instead of a minute required by the automatic approach to register the entire brain vasculature.

We then investigated this comparison for inter animal registration. Predictions from our automatic approach between different animals were shifted by $164 \pm 78 \mu\text{m}$ (ranging from 139 ± 66 to $188 \pm 83 \mu\text{m}$) compared to the first annotator and by $220 \pm 104 \mu\text{m}$ (ranging from 163 ± 76 to $266 \pm 129 \mu\text{m}$) compared to the second. The two experts estimations were misaligned by $259 \pm 102 \mu\text{m}$ (ranging from 214 ± 87 to $297 \pm 114 \mu\text{m}$), yielding again an higher inter-expert discrepancy than when compared with the BPS result.

Ultrasound Localization Microscopy (ULM) allows to evaluate registration error at micrometric scale

In order to assess the accuracy of the vascular registrations at even smaller scale, we further measured the remaining misalignment using super resolution images after repositioning with the BPS as described in Materials and methods section. One can barely notice misalignment when looking at Power Doppler images of a pair of acquisitions overlaid over each other (Fig. 5A) especially in direct registration trials where we expect lower misalignment. A few shifts in large vessels can be noticed in indirect registration acquisition which can be due to inherent differences in the vascular architecture of the animals, but the image resolution does not allow a fine estimation of the misalignment. On the other hand, after 3 minutes of acquisition about 3.10×10^5 bubbles were detected to reconstruct a super-resolved coronal and sagittal slices with $5 \mu\text{m}$ pixel size (Fig. 5B). The images corresponding to the local microbubbles density illustrate the increase in spatial resolution on the whole image. These superresolution images allow us to provide an accurate estimation of the BPS remaining misalignment between a pair of acquisitions. Local vasculature details can be appreciated within zoomed box regions (Fig. 5C). For each pair of acquisitions the displacement map was averaged over the entire brain region to estimate the registration error for this pair. With a total of 15 pairs of coronal acquisition (X,Z direction) and 7 pairs of sagittal acquisitions (Y, Z direction) we estimated the intra-animal vascular registration error to $(44 \pm 32 - 31 \pm 23 - 21 \pm 10) \mu\text{m}$ respectively in X (lateral) direction, Y (elevation) direction and Z (axial) direction. The 12 pairs of coronal acquisitions and the 10 pairs of sagittal acquisition after indirect registration on a reference vasculature from different animal show average inter-animal registration error of $(74 \pm 38 - 96 \pm 69 - 50 \pm 29) \mu\text{m}$ respectively in X (lateral) direction, Y (elevation) direction and Z (axial) direction.

Task evoked activation maps

One of the goals of this study was to validate the fully automatic neuro-navigation approach proposed here for functional ultrasound imaging application. Here, the goal is to perform functional mapping of the brain activation in a 2D plane containing two chosen regions of

interest without the intervention of a neuroanatomist expert. First, the BPS was used to provide whole brain annotation in the experimental framework (Fig. 6A) enabling whole brain exploration and real-time anatomic identification. Leveraging this asset, we automatically targeted an oblique plane encompassing both the visual cortex in one hemisphere and the somato-sensory area in the second hemisphere as labelled in the Allen Atlas (Fig. 6A red box). After automatically moving the probe to this oblique slice we then recorded transcranial Power Doppler images during successively right whiskers stimulation and left visual stimulation in anesthetized conditions. In $n=4$ mice for both stimuli, we obtained high and significant (p -value = 0.00001 after stringent Bonferroni correction for multiple analysis) z-score values (Fig. 6B). Still leveraging the BPS, the structure contours are automatically overlaid over Power Doppler images and z-score maps with a good agreement between the activated regions and the targeted functional regions. It validates the targeting and automatic probe positioning of the BPS approach to enable functional ultrasound imaging in complex planes for non-experts.

Functional connectivity analysis

Finally we demonstrated our neuro-navigation approach capability for the acquisition and analysis of functional connectivity.

Fig. 7A shows seed-map overlaid onto the baseline Power Doppler images and the functional regions (from Allen atlas) automatically aligned. The seed region is indicated with a different color. At Bregma -1.76 mm position we observed high correlation within hippocampal formation and a good overlap between the automatically aligned regions and the seed map. Cortical bilateral connections are also observed at Bregma -1.26 mm and Bregma -0.76 mm, as well as deeper structures such as thalamus or olfactory areas.

The connectivity matrix over 50 regions (for a single slice) extracted from the Allen atlas allows to observe robust connectivity between interhemispheric functional regions as well as higher connectivity within main functional areas (Fig. 7B) as previously observed in fUS connectivity analysis with manually positioned regions in the literature.

Discussion

Functional ultrasound with ultrafast Doppler imaging is capable of mapping and recording blood flows variations and allows for functional activation mapping and connectivity analysis. Here we demonstrate that 3D power Doppler vascular fingerprints can be automatically registered on the fly to provide automatic neuro-navigation for live probe positioning and atlas-based data analysis. Using angiographic images obtained with Ultrasound Localization Microscopy, we demonstrated the vascular registration accuracy to be less than a hundred of microns i.e. the

size of the Power Doppler pixel and more accurate than the inter-experts manual landmarks annotations.

The BPS appears to rely mostly on larger vessels distributed throughout the brain rather than small vessels, which is consistent with the fact that smaller vessels may be more subjects to change between individual mice. We believe that local variations between vessel architecture cancel out overall in the whole brain which allows the registration to remain accurate in similar strains of mice. These invariants of vascular shapes versus scale relationships will be further studied in subsequent work.

We demonstrated the use of the BPS for functional ultrasound imaging in complex oblique planes automatically with a good match between activated areas and atlas-based structures as well as functional connectivity matrix assessment with automatic ROIs extraction for any plane.

Our study has several limitations. First of all, all mice used in this study are from the strain C57BL/6 and aged between 7 and 14 weeks. The ability to use the same vascular reference for mice with different ages, for different models, or pathologies has to be further investigated and it might be needed to use a different ad-hoc vascular template adapted to each category. On the processing side, the registration process was also limited to a simple affine registration algorithm [21]. However, it would be straightforward to include non-rigid deformable registration based on deformable demons [25,26], B-spline [27] or thin-plate spline [28] registration for instance which might be required in more complex applications such as neurodevelopmental studies, or to take into account large brain deformation for instance due to a craniotomy (brain shift), large cerebral tumor growth or cerebral oedema/brain swelling. This approach could also be required for animal models with less genetic homogeneity which could yield more diverse brain and vasculature shapes across subjects. This would certainly be the case for functional ultrasound imaging with BPS in primate [29] or humans in the case of intraoperative [30,31] settings or neonate imaging [32,33] where there can be a high natural variability between subject brains.

Although recent promising advances in the development of 2D Matrix arrays or Row Columns arrays have been proposed for full 3D functional ultrasound imaging [34,35,36], their sensitivity remains limited which still precludes transcranial imaging in mice. The proposed approach still remains valid for volumetric imaging even if the need for perfectly positioning the probe prior to the acquisition is reduced as there remain more possibilities to register volumes in post-processing.

Another application of the on the fly BPS approach is the use of the Doppler images and a robotic motorized platform to allow guidance of tools deep in the brain such as needles for micro injections, micro or array electrodes for recording or optical fibers for optogenetic stimulation in deep areas. This could also be a promising tool for human surgery and the positioning of electrodes for deep brain stimulation especially in conjunction with live functional ultrasound imaging.

Finally, the super resolution vascular images were used here only as a mean to investigate the registration accuracy but they could one day be used as a second step to further refine the registration to tens of micrometer scale, enabling extremely precise neuronavigation in depth in a unique way.

The brain vascular GPS approach presented here enables on the fly complex brain navigation without neuroanatomic expertise in the context of functional ultrasound acquisition and data analysis with more accurate positioning and identification of regions of interest. It could help with the standardization of acquisitions in standardized planes to allow the building of large, reproducible and reliable fUS datasets and studies. Beyond mice studies, the BPS proposed here can be generalized to many other animal models including rats, ferrets, marmosets, non human primates as well as to clinical applications such as transcranial imaging [17], neurosurgery guidance or neonate functional imaging.

Data availability

Data supporting the findings of this study are available in the framework of an official collaboration between academic institutions.

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Author contributions

M.N., B.O., M.T. and T.D. designed the experiments. M.N., J.F., and N.I. performed the experiments. M.N., B.O. and T.D. developed the algorithms. M.N., S.P., and J.F. analysed the data. All the authors discussed the results and wrote the paper.

Competing interests

Mohamed Nouhoum is PhD student in Physics for Medicine Lab and funded by Iconeus. Jeremy Ferrier and Bruno-Félix Osmanski are employees of Iconeus. Thomas Deffieux, Bruno-Félix Osmanski, and Mickael Tanter are co-founders and shareholders of Iconeus.

Figures and Table legends

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Table 1 : Landmarks-based comparison between automatic registration predictions and neuroanatomists experts manual annotations of these landmarks. 20 registrations operations were performed for both intra-animal and inter-animal acquisitions between pair acquisitions (inter- or intra-animals). For each pair, the automatic registered data was resampled in the reference dataset space and two neuroanatomists experts were asked to annotate four landmarks within the two datasets. Individual landmark 3D distance shifts between registration prediction and expert annotation were averaged over the 20 estimations, as well as the overall shift. Automatic registration (AR) was compared to individual expert annotation and the two experts annotations were compared to each other. Automatic registration predictions were globally shifted by $120 \pm 84 \mu\text{m}$ related to the first expert

annotation and by $130 \pm 82 \mu\text{m}$ related to the second whereas inter-annotator shift was globally estimated to $215 \pm 87 \mu\text{m}$ for inter-animal datasets registration. The same shifts are estimated to respectively $164 \pm 78 \mu\text{m}$, $220 \pm 104 \mu\text{m}$ and $259 \pm 102 \mu\text{m}$ for inter-animal datasets registration.

Figure 5 : Registration accuracy estimation based on super-localization imaging.

Successive and time-delayed registrations are used to position the probe and image the same coronal or sagittal slice. Shifts from reconstructed images from several trials can be estimated as 3D translations enabling the evaluation of the registration process. **A.** Power Doppler images from a pair of acquisitions are overlaid both in coronal and sagittal directions and for both intra-animal and inter-animal data registrations. Misalignment can not be correctly estimated with this level of details ($100 \mu\text{m}$ resolution). **B.** Corresponding super-Localization images as microbubbles density reconstructed with $5 \mu\text{m}$ pixel. Scale bar is $500 \mu\text{m}$. **C.** Zooming boxes showing finer local misalignment. Displacement map was computed as 2D translations between a pair of images and averaged over the whole images and over all the pair of acquisitions to evaluate intra-animal and inter-animal data registration accuracy in the 3 space directions as Δx (lateral error from coronal acquisitions), Δy (elevation error from sagittal acquisitions) and Δz (axial error from both coronal and sagittal acquisitions). Scale bar is $200 \mu\text{m}$ within zoomed boxes.

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Figure 7 : Transcranial functional connectivity analysis with BPS and atlas-based segmentation. **A.** Seed-based analysis. The grayscale images represent the baseline Power Doppler images. Seed regions are indicated in magenta. Color-coded correlation maps are overlaid to baseline images for each of the selected seed regions. Automatically aligned anatomic delineations from the Allen CCF are shown in green for reference. **B.** Connectivity matrix analysis. BPS enabled anatomic boundaries delineation and data extraction from about 70 regions for each of the 3 imaging planes. Regions are overlaid onto the baseline Power Doppler images. Connectivity matrices were computed based on pairwise correlations between signals from individual regions. The matrices show color-coded correlation coefficients.

Figure

1

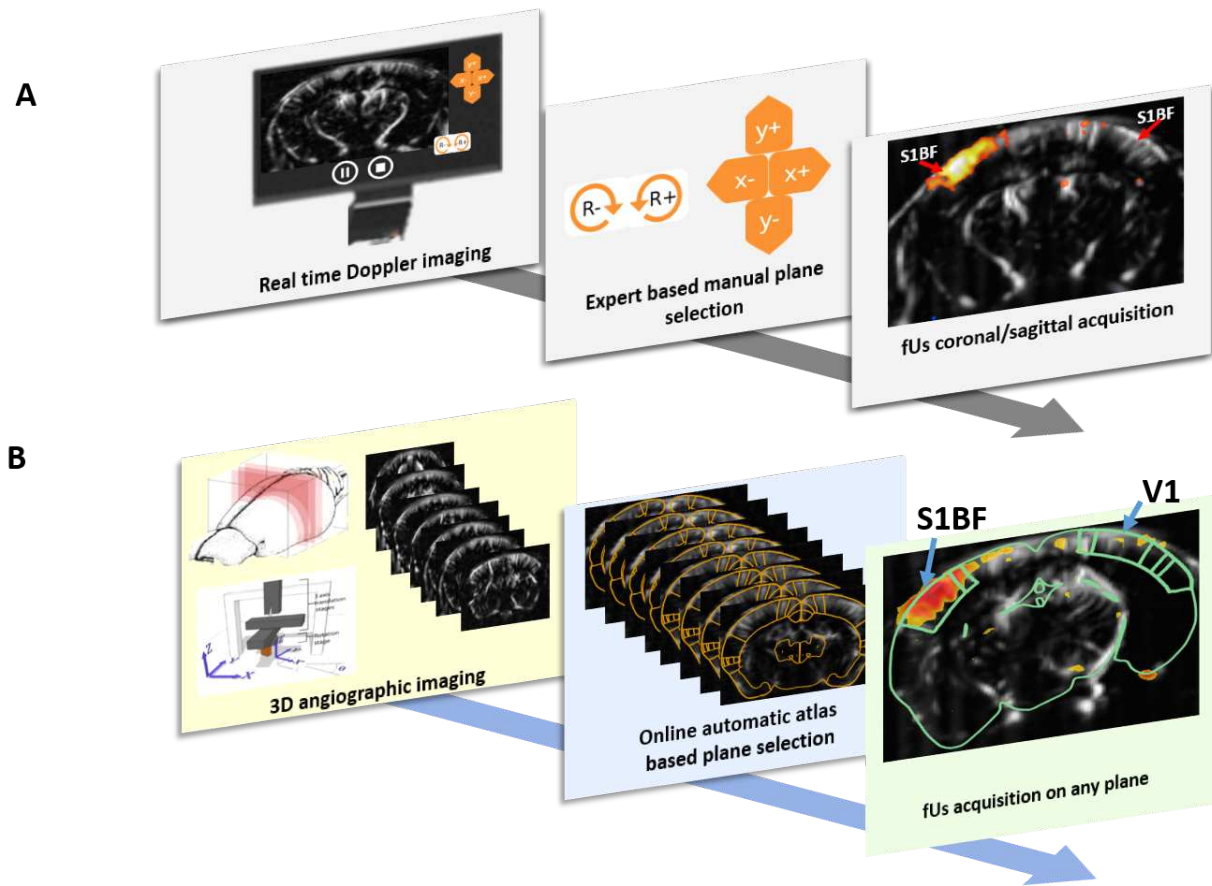


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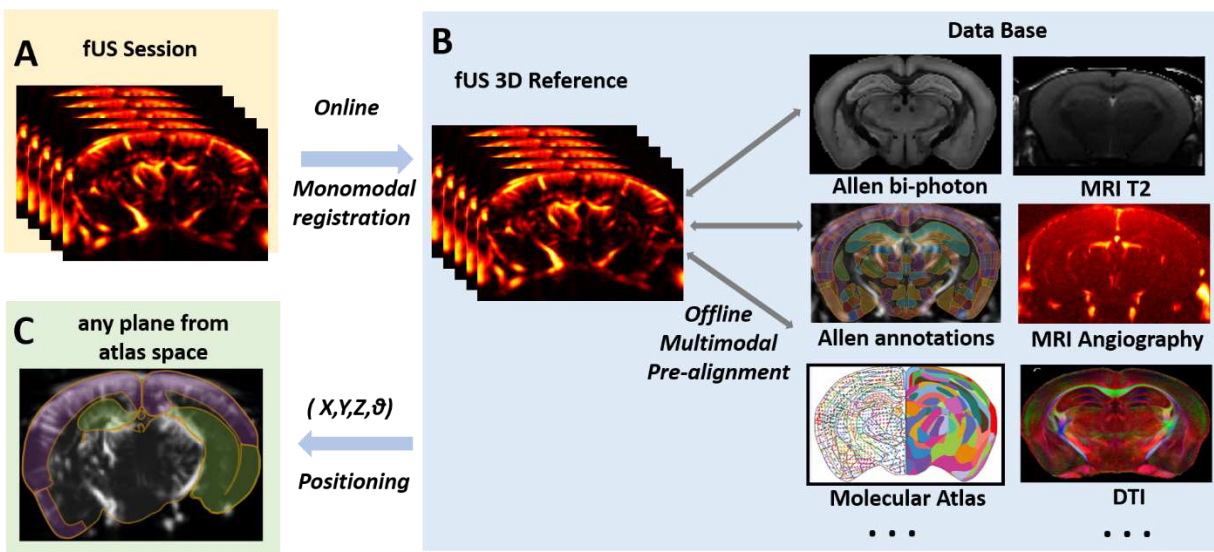


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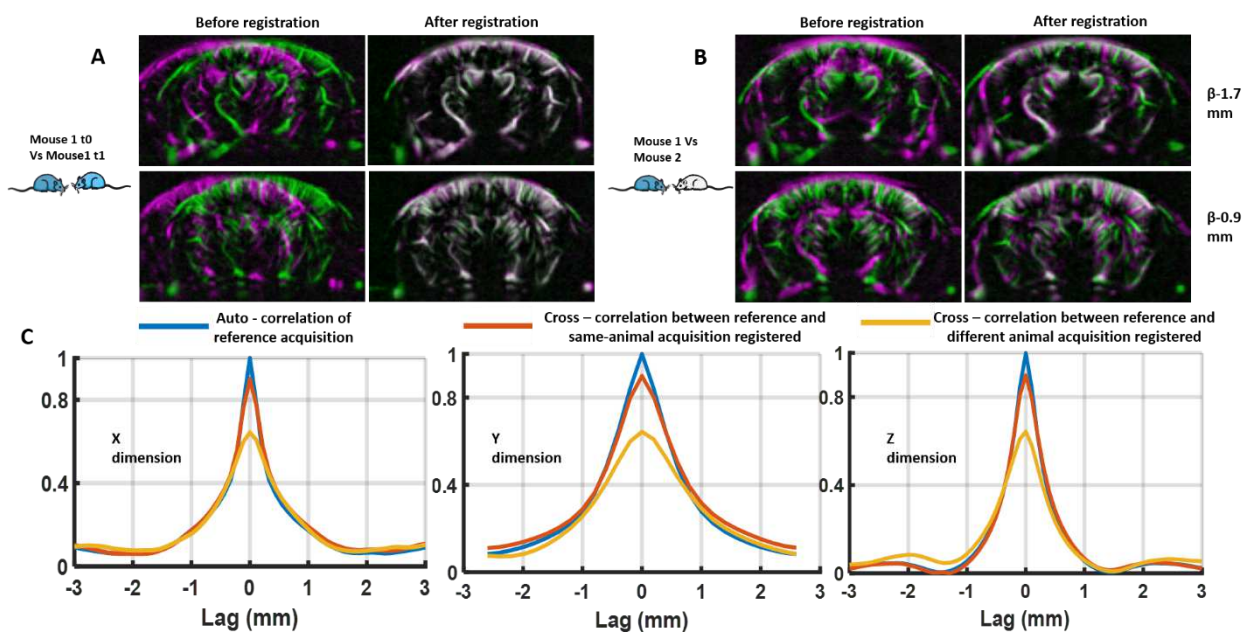


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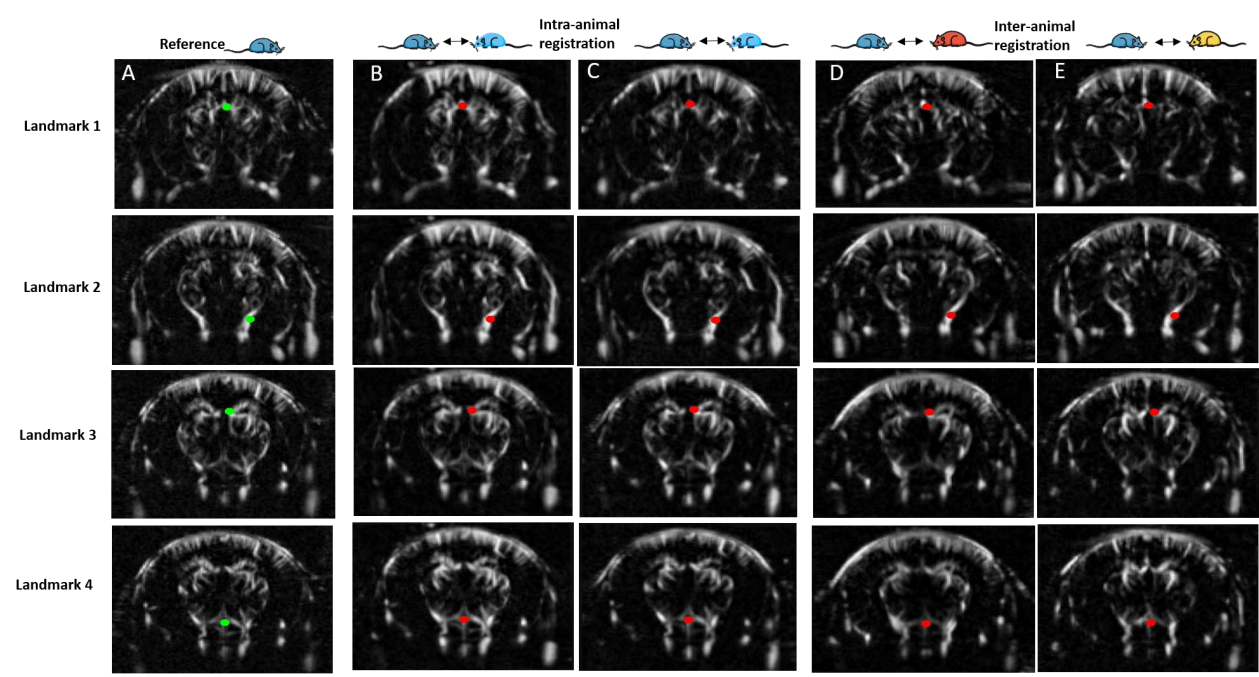


Table 1

	Intra-animal registrations (n=20)			Inter-animal registrations (n=20)		
	AR Vs Expert1	AR Vs Expert2	Expert1 Vs Expert2	AR Vs Expert1	AR Vs Expert2	Expert1 Vs Expert2
Landmark 1 (μm)	158 ± 94	155 ± 90	184 ± 94	188 ± 83	266 ± 129	297 ± 114
Landmark 2 (μm)	78 ± 58	140 ± 90	191 ± 76	152 ± 78	207 ± 97	214 ± 87
Landmark 3 (μm)	154 ± 82	105 ± 70	253 ± 101	178 ± 81	246 ± 82	269 ± 113
Landmark 4 (μm)	88 ± 69	118 ± 74	233 ± 57	139 ± 66	163 ± 76	255 ± 75
All (μm)	120 ± 84	130 ± 82	215 ± 87	164 ± 78	220 ± 104	259 ± 102

Figure 5

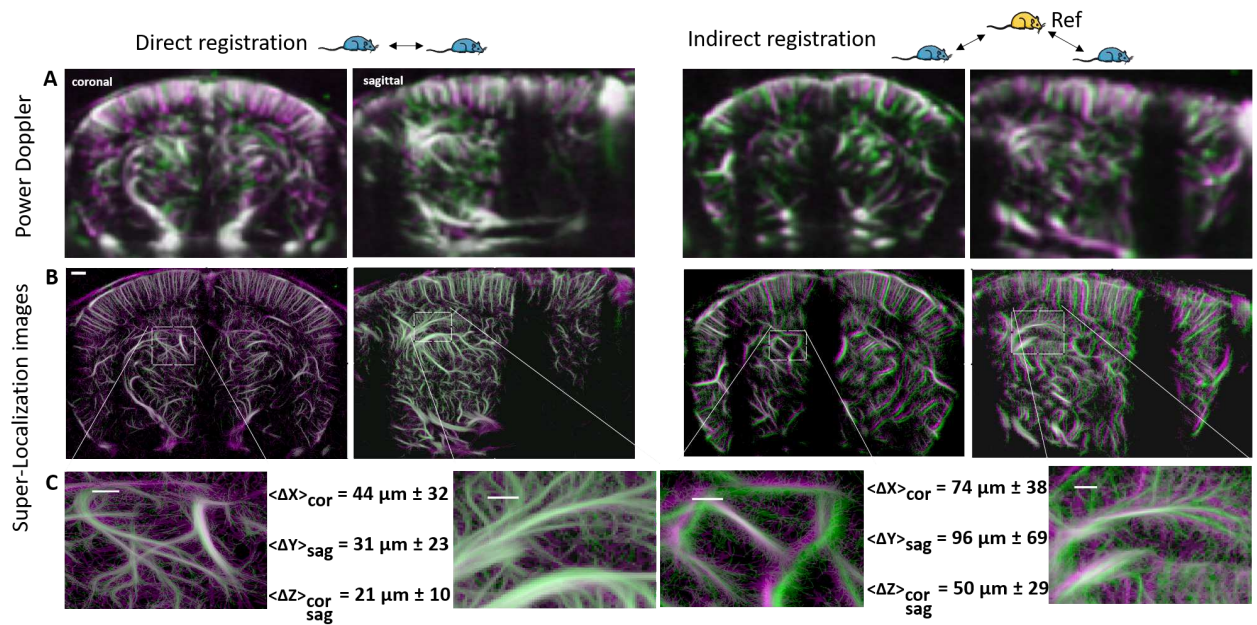


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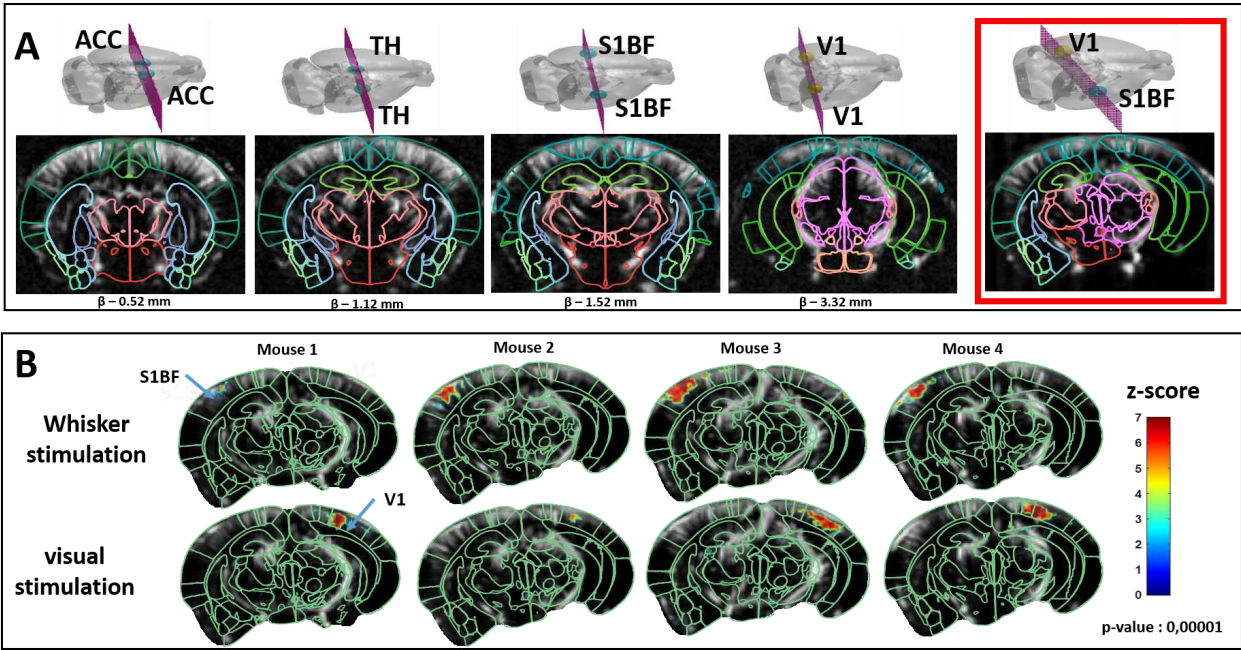
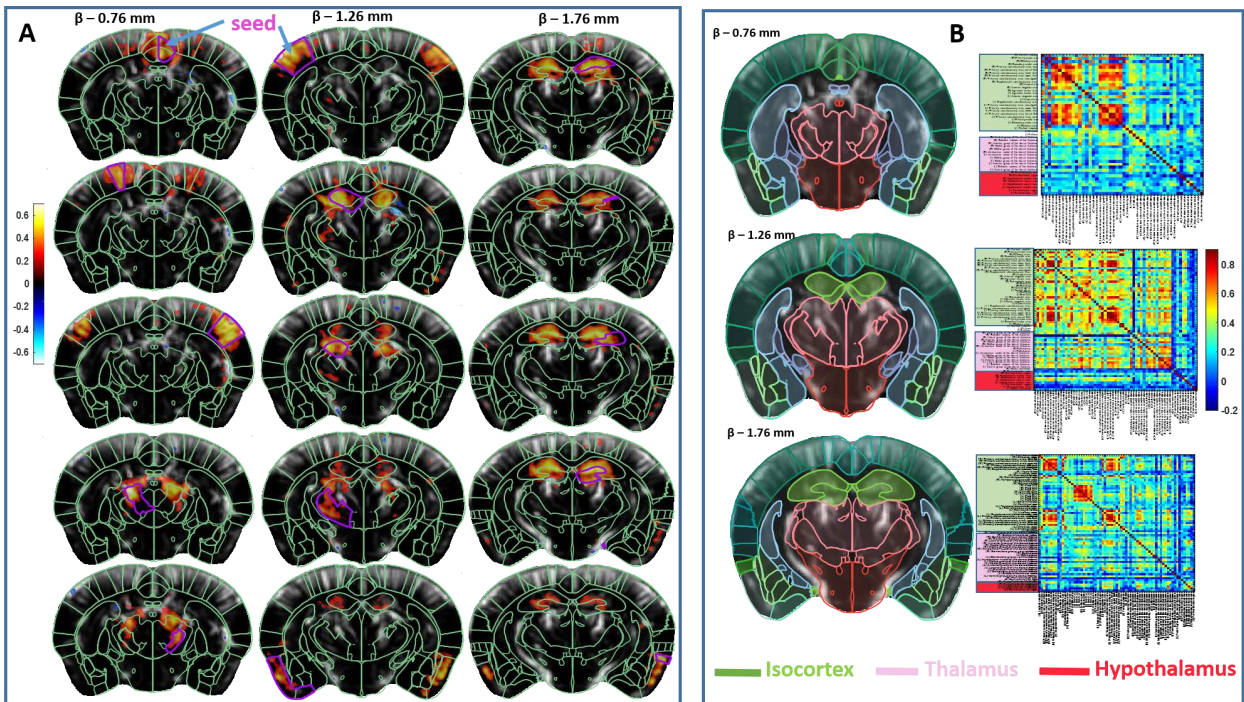


Figure 7



Figures

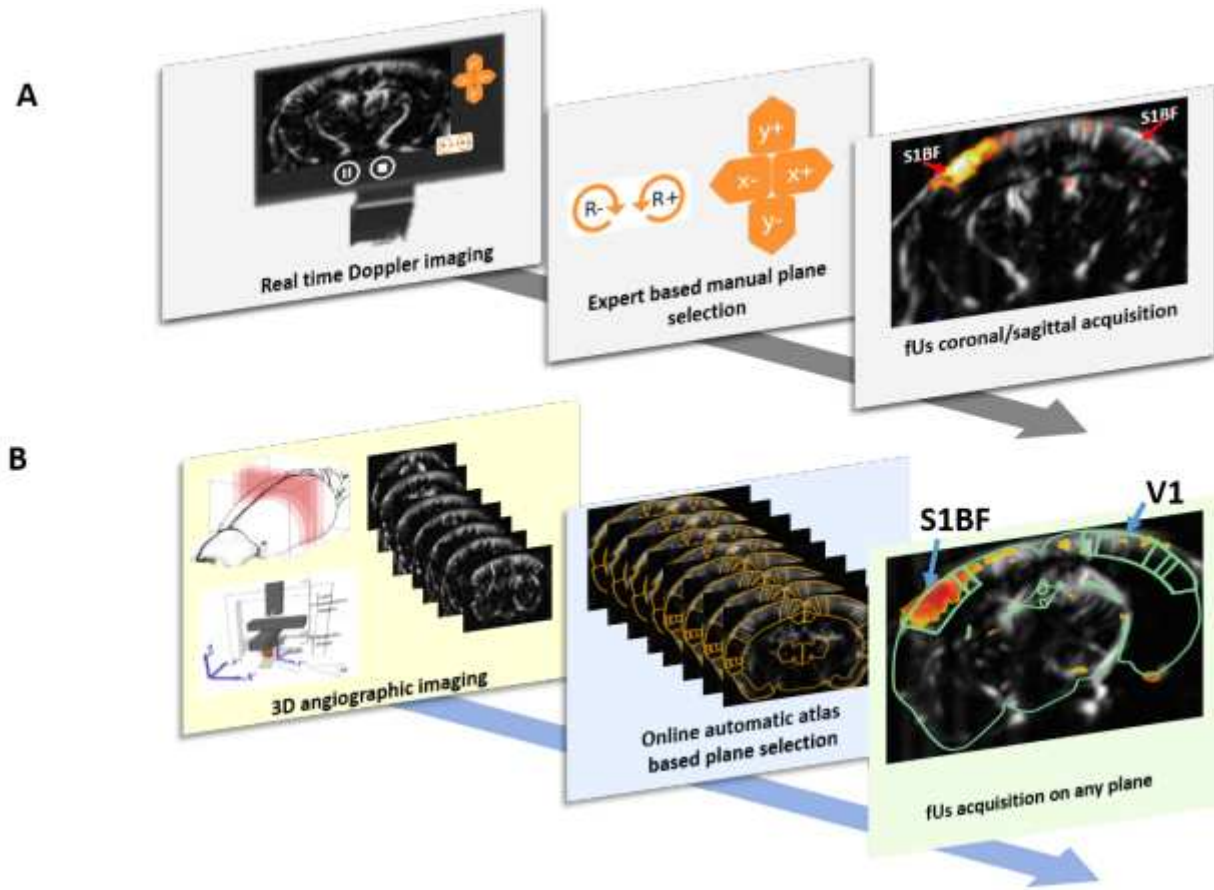


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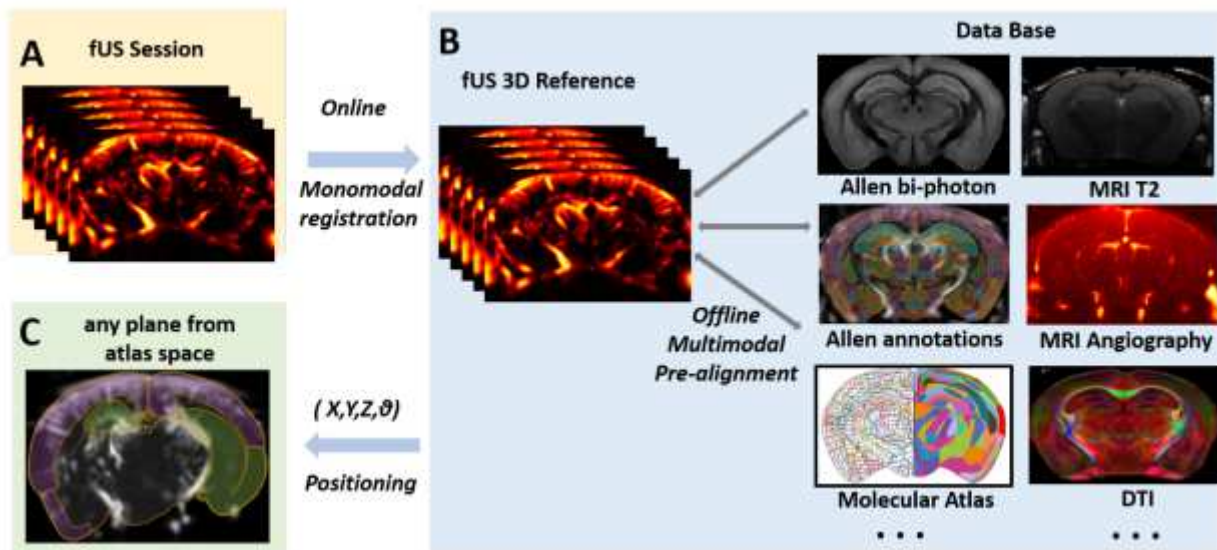


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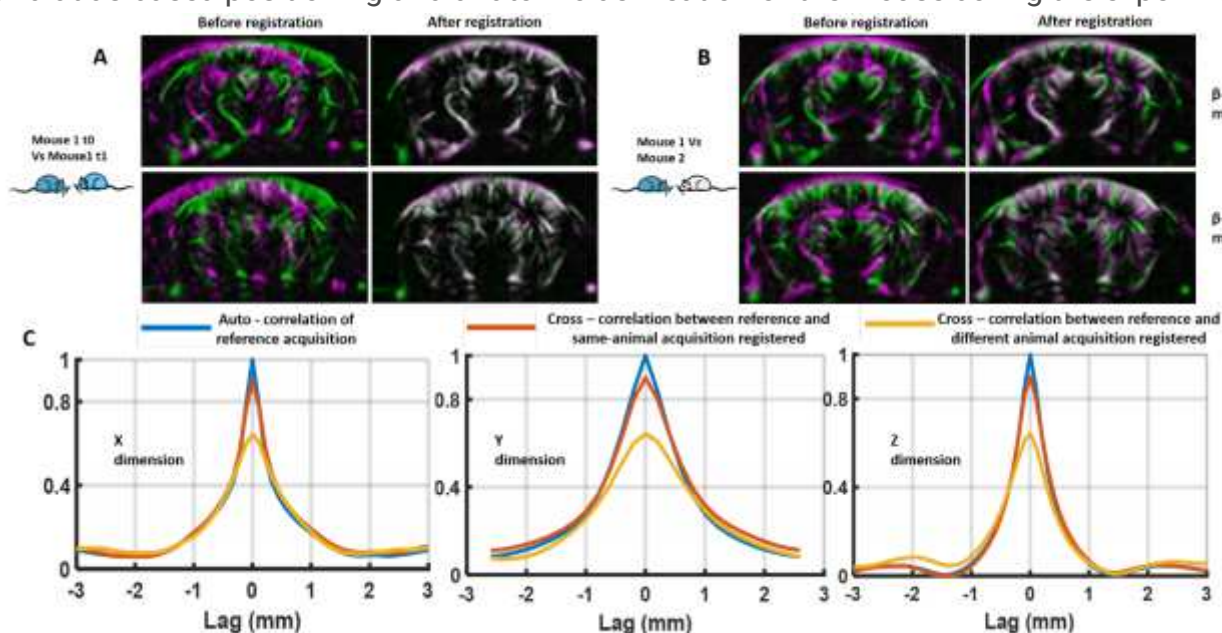


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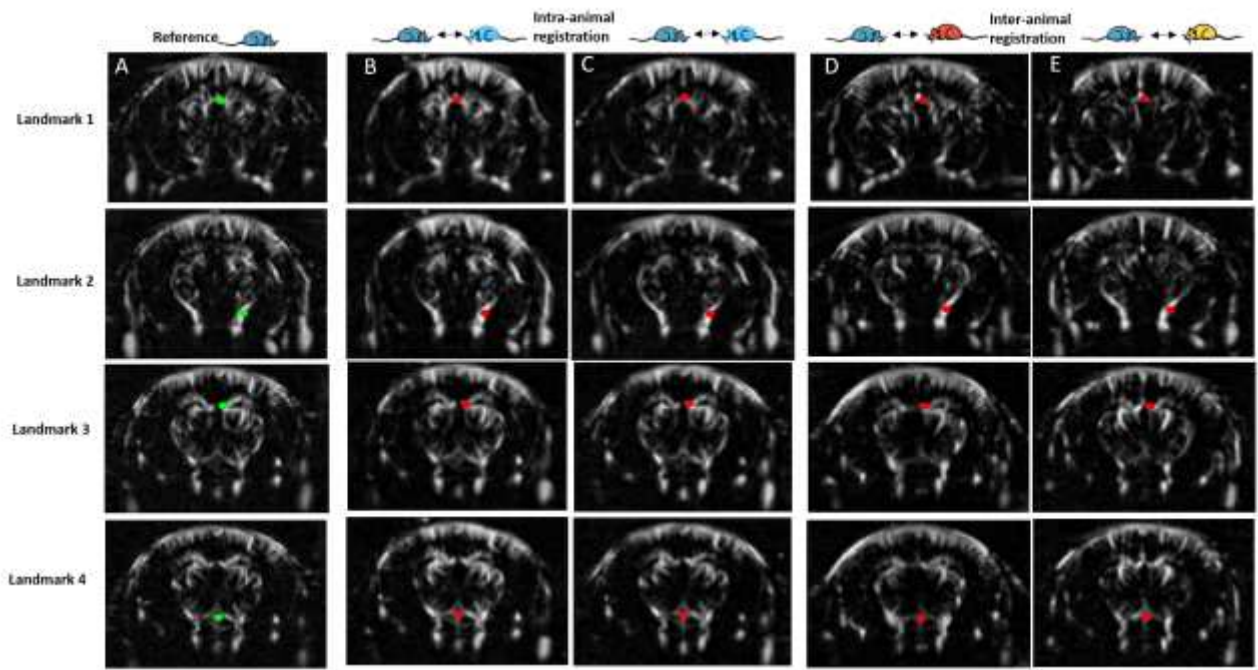


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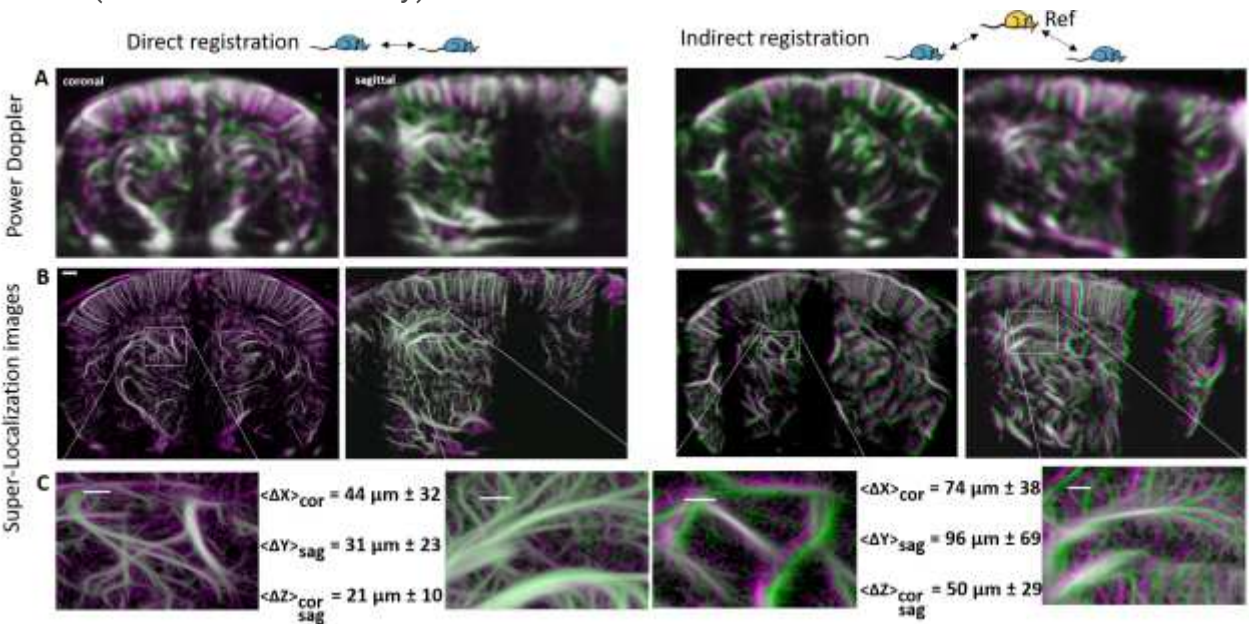


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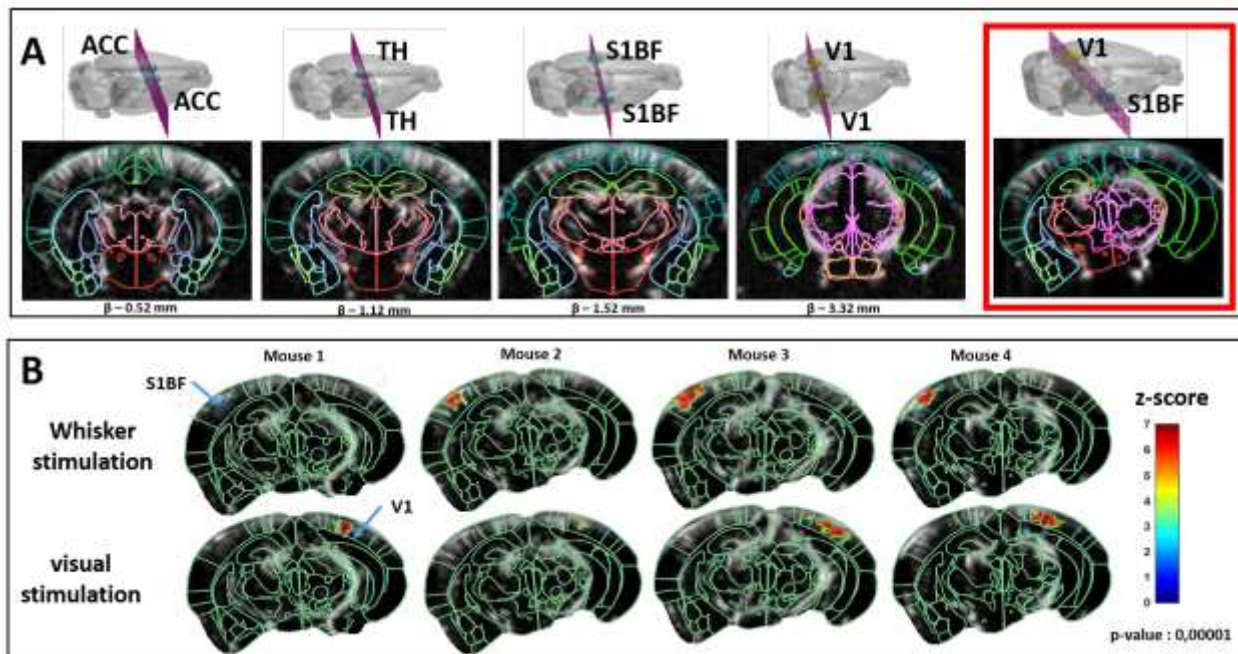


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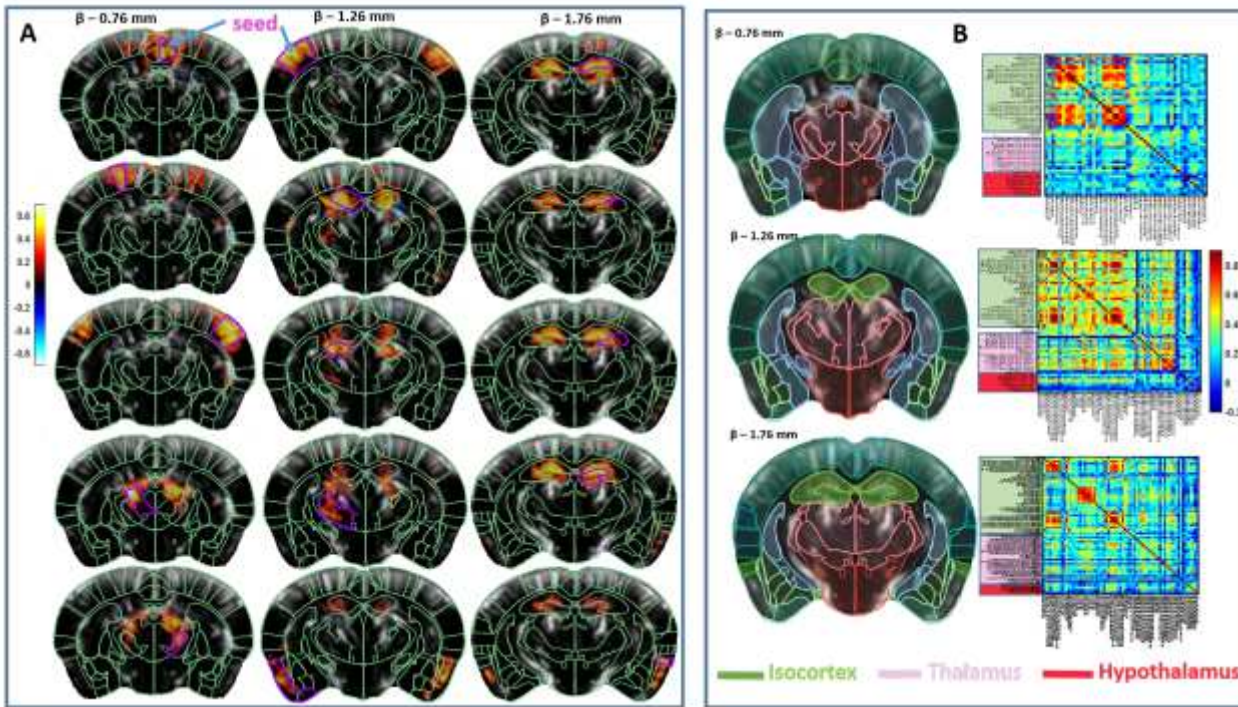


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