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High Resolution Ex Vivo Structural MRI Imaging & Analysis Protocol

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Martinos Center for Biomedical Imaging

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At the Martinos Center for Biomedical Imaging, we are continuously developing cutting-edge techniques to enable high-resolution ex vivo MR Imaging data and novel data processing techniques. We form one site of a multi-site project with the goal to perform an imaging & analysis pipeline towards generating a cell census for Brodmann's Area 44/45 in the human brain. Magnetic resonance imaging (MRI) is used to establish a macroscopic reference coordinate system of laminar and cytoarchitectural boundaries. Cell counting is obtained with both traditional immunohistochemistry, to provide a stereological gold-standard, and with a custom-made inverted confocal light-sheet fluorescence microscope (LSFM) for 3D imaging at cellular resolution. Finally, mesoscale optical coherence tomography (OCT) enables the registration of the distorted histological cell typing obtained with LSFM to the MRI-based atlas coordinate system. This protocol outlines the steps involved in generating the MRI data for a reference system of the cell census.

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1. Sample Preparation for Scanning

Beginner note: Pia should be removed for the surface of the hemisphere carefully (so as not to tear the tissue). This greatly reduced the number of bubbles in the pack and therefore in the MRI scan data.

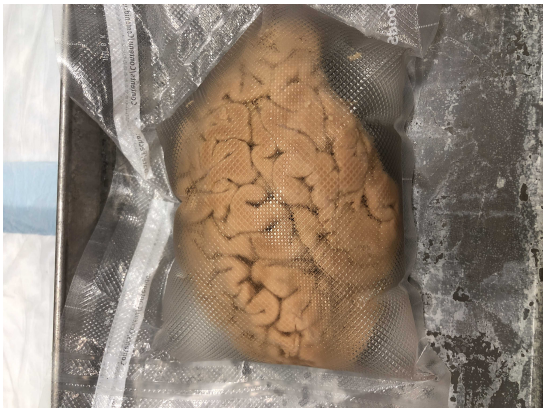
Required Equipment:

- Sample
- Packing Fluid (Usually Fomblin with no MR signal, or PLP)
- Metal tin
- Large roll of Food Saver Bags
- Aluminum Foil Folded into a long rectangular shape
- Sealer
- Funnel

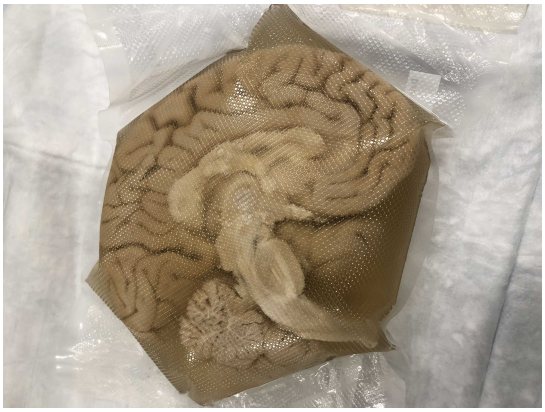
Cut around 1.5 ft off a roll of Food Saver bags (around 2 x the height of the sample). A foot sealer is used to create 3 parallel seals, we take care to ensure the seals are smooth and high quality - that is to say there are no areas where the plastic is burned and no crinkles are present. Next, we use the sample as a template and mark off with Sharpie on the bag the general outline of the sample. Then, the foot sealer is used to create a second set of seals, at ~30 degree angles sealing off the two bottom corners of the bag. We slide the specimen into the bag, with the cortex against the smooth side of the bag to minimize damage and bubbles. Next, we insert a strip of aluminum foil into the opening of the bag and center it. We take care not to slice the sample with the foil. The foot sealer is used to create a set of seals near the top of the sample, at ~45 degree angles, sealing off the top two corners of the bag, except for where the foil strip is placed. Next, we remove the foil and pour in the scanning solution (PLP or fomblin, depending on project requirements). As solution is poured in, the bag is manipulated and the sample is turned over to ensure any big bubbles escape - especially around the ventricles. We continue to add liquid and then take breaks to move the sample around and allow more air to escape. We make

sure to add enough solution so that it fills the canal where foil was placed before - this extra solution will be vacuumed out later. We create a fifth set of parallel seals at the opening of the bag and then use a dedicated tool to pierce a hole in one side of the bag and mark this area with a Sharpie. A vacuum beaker, vacuum nozzle and vacuum tube are then connected to a fume hood vacuum system. The vacuum tube is placed over where the bag is pierced. A few iterations of the following are performed: we vacuum air out of the pack, seal the hole with tape, massage the sample to release bubbles from the sulci and move them up into the vacuum area before vacuuming them out. After doing this ~3 times, we will tape over the hole and leave the sample to sit for a few days in the refrigerator. Closer to the scan date, we remove the sample and perform a final vacuum. When we are confident that no bubbles are visible in the pack, we tape over the hole a final time and use the foot sealer to create a sixth set of seals which are below the piercing and parallel to the sample, sealing off the area where any air could creep back into the pack. Excess bag is trimmed off and the sample is left for 12 - 24 hours to heat up to room temperature before scanning.

Figure 1: Example images from a hemisphere packed in Fomblin (left) and PLP (right)



Medial view of packed hemisphere sample



Lateral view of packed hemisphere sample

A paper will be published soon on the specifics of these methods. For help in the meantime, feel free to reach out to researchers in the Laboratory for Computational Neuroimaging at MGH who will gladly share their resources. https://www.nmr.mgh.harvard.edu/lab/lcn

2. Protocols for Acquiring High-Resolution Structural MRI

Our high-resolution data is acquired using a multi-echo fast low-angle shot (ME FLASH) sequence at either 120 um isotropic spatial resolution. Cases scanned before 2019 were routinely collected at 150 um isotropic spatial resolution. We acquire multiple flip angles ranging from 10 to 50 degrees during the experiment. Development towards the sequences for high resolution structural MR imaging which we use in this project are built on many years of exvivo imaging research[1,2].

For correcting B0 inhomogeneities, we collect a 2D-encoded B0 field map, and a standard gradient-echo field map consisting of two gradient echoes. which estimates the amount of inhomogeneity at every voxel and in turn gives us a measure of displacement at every voxel of the high-resolution isotropic ME FLASH scans.

Finally, B1+ variation is estimated by acquiring multiple single echo FLASH sequences with short TE (2.7ms), long TR (5 s), flip angles varying from 20° to 340°, FOV = 192 x156mm, matrix =96x78, and slice thickness = 2 mm.

Attached please find a protocol PDF with all scan parameters from a recent sample scan.
Example_protocol.pdf

3. Imaging Hardware

MRI Scanner:
At the Martinos Center, we have a research-dedicated 7 Tesla Magnetom Terra (Siemens) MRI. Search for Bay 5 on this webpage for more information on the scanners we use:
https://www.martinos.org/core-services/magnetic-resonance-imaging/

"This laboratory supports an ultrahigh-field 7 Tesla whole-body MRI with 70 mT/m (200 T/m/s max slew rate) gradient set (SC72B) and 32 RF receive channels. The 7T whole body magnet (90 cm magnet ID) was built by Magnex Scientific (Oxford, UK). Siemens provided the conventional MRI console, the gradient and gradient drivers, and the patient table. The system is shielded by 460 tons of steel. Integration of these components and the design and construction of RF coils were performed jointly by MGH and Siemens personnel. With its high-performance gradient set, the system can provide better than 100 µm resolution and ultra-fast EPI readouts for reduced image distortion. The system uses a home-built 32-channel or 8-channel head array coil for human imaging. A selection of specialized coils is also available forex vivoMR microscopy as well as primate imaging. The system has multinuclear imaging capability, and coils for 31P and 13C are available. The system has been upgraded by Siemens to contain 8 independent 1kW transmit channels capable of simultaneous parallel excitation with different RF pulse shapes for B1 shimming and/or parallel transmit methods such as transmit SENSE. The 7T scanner environment includes a visual display system and a button box for acquiring subject responses in the scanner. A MedRad power injector is installed in the Bay for the injection of gadolinium contrast agents."

Custom-built 32 Channel coil for ex vivo samples:
We use a custom-built 32 channel head coil for scanning our ex vivo samples[3]

An excerpt from the paper to describe the coil is included here:

"The coil former (Fig. 2c) consists of two halves and encloses the brain holder. The receive array coil consists of 31 detectors (Fig. 2a), with 15 elements on the top half (diameter = 5.5 cm) and 16 on the bottom half (diameter = 8.5 cm). Coil elements were constructed using 16 AWG wire loops17, each with four or five evenly spaced capacitors (Supplementary Fig. 1). All elements were tuned to 297.2 MHz and matched to a loaded impedance of 75 Ω to minimize preamplifier noise. Preamplifier decoupling was achieved with a cable length of 6 cm. Preamplifiers were placed directly on the coil elements, yielding a substantial reduction in cable losses compared to a previous 30-channel ex vivo brain array18. The active detuning circuit was formed across the match capacitor using an inductor and PIN diode.

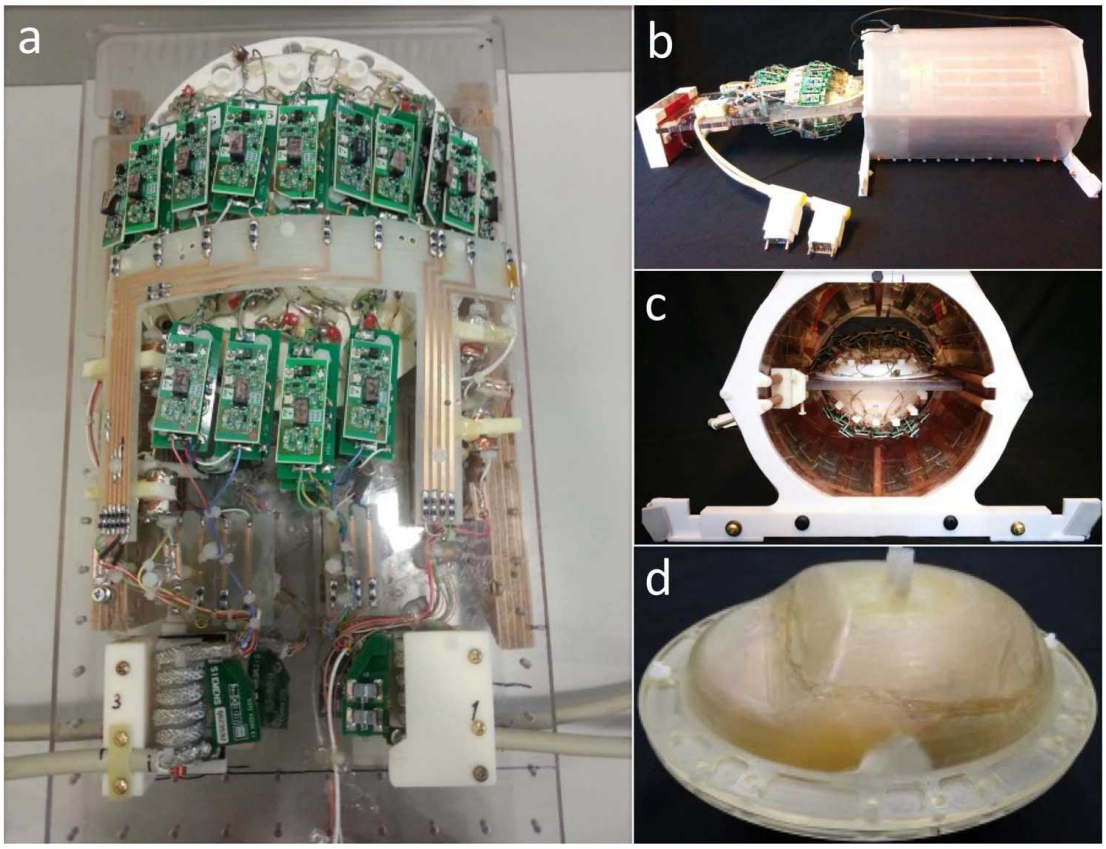
Tuning, matching, and decoupling of neighboring elements was optimized on the bench with a brain

sample immersed in periodate-lysine-paraformaldehyde (PLP) solution. Because coil loading varies with the fixative used, the coil must be tuned and matched on the bench using a brain sample with the correct fixative. (For example, testing can be performed with a brain sample immersed in PLP or formalin, but not the regular loading phantom comprised of water and salt). Loops tuned/matched on PLP showed unloaded-to-loaded quality factor ratio (Q-ratio) of QUL/QL = 210/20 = 10.5, corresponding to an equivalent noise resistance of 11 ohms for the loaded coil (Q = wL/R). By contrast, formalin is a less lossy fixative, giving a coil Q-ratio of QUL/QL = 210/60 = 3.5, corresponding to an equivalent noise resistance of 4 ohms.

A shielded detunable volume coil (Fig. 2) was built for excitation, with the following parameters and features: band-pass birdcage, diameter 26.7 cm, and an extended length of 32 cm to accommodate brain samples of larger dimensions. For the detuning circuit we used diodes in every leg of the birdcage. These diodes are powered with the high-power chokes, which can withstand high voltage and short duration inversion pulses.

In summary, this coil system incorporates an improved mechanical design, preamps mounted at the coil detectors, and an extended transmit coil design capable of producing high-power pulses."

Figure 2:Custom Head Coil Construction



4. Data Processing & Distortion Correction

Offline Recon

Here is an excerpt from a paper to describe the custom reconstruction of data collected in this protocol:

"The size of the k-space data exceeded the storage capacity of the RAID provided by the scanner's image reconstruction computer. The image reconstruction also required more RAM than what was available. We, therefore, implemented software on the scanner to stream the data directly via TCP/IP to a server on an external computer added to the scanner network, which saved the data as they were received. Because of additional limitations related to the total size of the raw data for any single scan, as dictated by the imager RAID size, we also divided each acquisition into segments. The server on the external computer stored the data as they were acquired, creating date stamps for every k-space segment.

After the scan was completed, the streamed k-space data were transferred to a computational server where we ran custom software to stitch together the segments, reconstruct the images for each channel (via a 3D FFT on each volume per channel20), and combine the images derived from the 31 channels via the root-sum-of-squares of the signal magnitudes at each voxel. These signal magnitudes were channel-wise decorrelated using a covariance matrix of the channels' thermal noise." [3]

B0 Distortion Corrections

2D-encoded B0 field map estimates the amount of inhomogeneity at every voxel and in turn gives us a measure of displacement at every voxel of the high-resolution isotropic ME FLASH scans. A group sparsity-based edge-preserving intensity correction algorithm was used to correct for B0 distortions[4]. The method uses the field map and all the FLASH images jointly to perform the corrections.

** A paper on the B0 distortion correction will be submitted in 2021. At that time we will link that paper here.

B1 Distortion Corrections

We estimate the flip angle variation due to B1+ distortion by fitting the multiple flip angle single echo FLASH with a sinusoid and estimating its frequency at each voxel. The multiplicative bias in our flip angle measurements can be calculated from the frequency estimate. The flip angle bias map is then used to correct the flip angle value at each voxel location. We fit T1 tissue parameter using a dictionary look-up procedure and the corrected flip angle map.We synthesized new FLASH MRI scans with the estimated T1 using the MRI physics forward model for FLASH MRI contrast to remove variations caused by spatially non-uniform B1+ field. [5,6]

** A paper on the MR acquisition and reconstruction of data will be submitting in October 2021. At that time we will link that paper here.

5. Vessel and Laminar Layer Segmentations

Please see this webpage for detailed instructions on how we segment the MRI data using our lab-developed software FreeView: https://sites.google.com/view/mgh-lcninternship/for-interns/research-project/infrasupra-help?authuser=0

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