Technical Note

Dedicated container for postmortem human brain ultra-high field magnetic resonance imaging

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\textbf{A B S T R A C T}

\textbf{Background:} The emerging field of ultra-high-field MRI (UHF-MRI, 7 Tesla and higher) provides the opportunity to image human brains at a higher resolution and with higher signal-to-noise ratios compared to the more widely available 1.5 and 3T scanners. Scanning postmortem tissue additionally allows for greatly increased scan times and fewer movement issues leading to improvements in image quality. However, typical postmortem neuroimaging routines involve placing the tissue within plastic bags that leave room for susceptibility artifacts from tissue-air interfaces, inadequate submersion, and leakage issues. To address these challenges in postmortem imaging, a custom-built nonferromagnetic container was developed that allows whole brain hemispheres to be scanned at sub-millimeter resolution within typical head-coils.

\textbf{Method:} The custom-built polymethylmethacrylaat container consists of a cylinder with a hemispheric side and a lid with valves on the adjacent side. This shape fits within common MR head-coils and allows whole hemispheres to be submerged and vacuum sealed within it reducing imaging artifacts that would otherwise arise at air-tissue boundaries. Two hemisphere samples were scanned on a Siemens 9.4T Magnetom MRI scanner. High resolution T2\textsuperscript{*} weighted data was obtained with a custom 3D gradient echo (GRE) sequence and diffusion-weighted imaging (DWI) scans were obtained with a 3D k-T-dSTEAM sequence along 48 directions.

\textbf{Results:} The custom-built container proved to submerge and contain tissue samples effectively and showed no interferences with MR scanning acquisition. The 3D GRE sequence provided high resolution isotropic T2\textsuperscript{*} weighted data at 250 \textmu m which showed a clear visualization of gray and white matter structures. DWI scans allowed for dense reconstruction of structural white matter connections via tractography.

\textbf{Conclusion:} Using this custom-built container worked towards achieving high quality MR images of postmortem brain material. This procedure can have advantages over traditional schemes including utilization of a standardized protocol and the reduced likelihood of leakage. This methodology could be adjusted and used to improve typical postmortem imaging routines.

1. Introduction

Postmortem magnetic resonance imaging (MRI) of human brain tissue improves the understanding of clinical MRI and provides insight into pathological data found via histological assessments of tissue (Mori et al., 2017; Seehaus et al., 2013). Human brain postmortem imaging has proven to be valuable due to the ability to scan for long periods (hours/days), compared to in vivo where patients are typically scanned for about one hour (Roebroeck et al., 2019; Augustinack et al., 2013). Longer scan times can lead to higher quality images through improved spatial resolution and greater signal-to-noise ratios (SNR) (Miller et al., 2012; Miller et al., 2011; Plantinga et al., 2016). Typically, ultra high field MRI (UHF-MRI, 7 Tesla and higher) examinations take place on

\textbf{Abbreviations:} CAM, computer-aided manufacturing; CNC, computer numerical control; cSt, centistokes; DREAM, dual refocusing echo acquisition mode; DWI, diffusion-weighted imaging; EPI, echo planar imaging; FA, flip angle; FoV, Field-of-view; GRE, gradient echo; LLDFE, low-density polyethylene; MRI, magnetic resonance imaging; PBS, phosphate-buffered saline; PMI, postmortem interval; PMMA, polymethylmethacrylaat; RF, radiofrequency; STEAM, STimulated Echo Acquisition Mode; SNR, signal-to-noise ratio; TA, total acquisition time; TE, echo time; TR, repetition time; UHF, ultra-high field.

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preclinical or animal MRI systems to utilize high field strengths and increased gradient performance, but are often limited to small tissue samples restricting the size of brain regions examined (Sengupta et al., 2018). Employing UHF-MRI systems to generate high resolution images of large human brain samples is an important step in investigating neuronal architecture which can then be translated to in vivo paradigms.

In preparation for scanning postmortem tissue, several aspects need to be addressed to obtain optimal data quality. The standard method of preserving large neuronal tissue is to contain it in fixatives such as formaldehyde that cross-links tissue proteins. This method, along with lowering of temperature, is an important factor in altering various MRI properties within tissue (e.g. T1 and T2 relaxation times) when compared to in vivo and in situ MRI (Dyrby et al., 2011). Because of these altered tissue properties scanning procedures must compensate with higher field and gradient strengths, close-fitting radiofrequency (RF) coils, optimized MR pulse sequences, and/or longer scan times to achieve high-resolution images (Roebroeck et al., 2019). Developing hardware for ex vivo imaging is imperative for optimal and more applicable postmortem imaging. Likewise, such hardware should aid in combating some of the main ex vivo MRI challenges including minimizing magnetic susceptibility gradients at tissue borders and removal of air bubbles from tissue that interfere with the MR signal.

The quality of in vivo brain scans are limited by a few factors that can be alleviated in postmortem acquisitions: the limited acquisition time, physiological noise from heartbeat and breathing, peripheral nerve stimulation, and specific absorption rate limits. Inhomogeneity caused by air cavities within the ear canals and sinuses are also a significant factor limiting the quality of in vivo scans. In order to obtain high quality images, a relatively homogeneous magnetic environment must be created over the entire sample, including at its borders. To achieve this for postmortem acquisitions, before scanning brains are typically submerged in a proton-free fluid such as Fluorinert™ or Fomblin®. Fluorinert, a perfluoropolyether fluorocarbon, avoids off-resonance distortions at tissue borders due to the fluids’ magnetic susceptibility being matched to that of brain tissue (Roebroeck et al., 2019). Fluorinert has shown to pose no risk to tissues’ histochemical properties even after a one-week immersion period (Iglesias et al., 2018). Although less optimal, submersion in phosphate-buffered saline (PBS), or formalin is sometimes performed. Prior to scanning, it is important to remove air bubbles from the surface, ventricles, sulci, and fissures, as trapped air bubbles will cause imaging artifacts. This can be done by gently agitating and rotating the tissue while having it completely submerged in liquid. Additional removal of air bubbles can be performed via a vibrating plate and/or placing the container inside a vacuum chamber. After scanning, Fluorinert™ can be stored and recycled for use during the next scanning session.

Within an MRI scanner, radio frequency receive coils made for certain areas of the body (e.g. head, chest, and knee coils) are used to measure the MR signal. Some studies have used custom-built receive coils specifically designed to image large postmortem samples at high resolution (Sengupta et al., 2018; Edlow et al., 2019; Fritz et al., 2019) although these methods are currently too financially and technically complex to become widespread common practice. Another study used a jug filled with formalin inside a head-coil to examine whole postmortem brains, but there was a large distance between the coils and the sample that limited the uniformity of the SNR profile, restricting image quality (Shatil et al., 2016). Although several studies have simply submerged large tissue samples in sealed plastic bags within MR head-coils with good results (Dyrby et al., 2011; Iglesias et al., 2018; Schmierer et al., 2007; Foxley et al., 2014) this leaves room for susceptibility effects caused by air–tissue interfaces while the possibility of leaks poses considerable risk to the tissue sample and the MRI scanner.

Lastly, MRI scanners are made by different vendors (e.g. General Electric, Siemens, Philips) and the head coils used may be made by diverse manufacturers, the same vendors, or third parties, which may not allow for the accommodation of all types of containers. Therefore, here we describe the manufacturing and use of a dedicated container for whole human brain postmortem imaging. The standard sized head shaped MRI container is capable of holding various submersion liquids and can be placed under vacuum pressure to minimize air bubbles while decreasing the distance between the coils and tissue for optimized positioning. We demonstrate the use of the container in a large bore 9.4T MRI system. This container optimizes MR imaging conditions for whole human brain postmortem imaging, while allowing efficient and flexible use in standard head-coils.

2. Methods

2.1. Construction of container

A container was developed in collaboration with the Instrument Development Engineering and Evaluation (IDEE) department at Maastricht University. The dimensions for the space inside a 7T 32 channel Nova Medical MRI head coil (Nova Medical, Wilmington, MA) where digitally rendered using Creo Parametric 3D Modeling Software 3.0 (Parametric Technology Corporation, PTC, USA) (Fig. 1B). A cardboard model with the gross dimensions of the container was laser cut and tested within multiple 7T and 9.4T head-coils available at the local MRI facility (Scannexus; Maastricht University campus; www.scannexus.nl). The container consisted from two halves (shells) and a lid milled from poly-methylmethacrylat (PMMA) using a Fehlmann P82 computer numerical control (CNC) milling machine over the course of 20 h with ESPRIT 2016 software (DP Technology Corporation, USA), a high-performance computer-aided manufacturing (CAM) system. After being shaped and smoothed the two shells were clamped and glued together using ACRI-FIX® 1S 0117, a solvent acrylic cement. The container was then dried at 70 °C for 18 h in a Memmert UN110, a universally applicable lab oven, to anneal the stress after milling, then cooled down to room temperature over a six-hour period. Finally, the container was smoothed and polished by hand in order to achieve complete transparency of the PMMA. The outside diameter is 177 mm and the length of the container is 245 mm (Fig. 1A).

A PMMA lid was constructed with a silicon sealing-ring on the bottom. Two polycarbonate 3-way stopcock Luer-lok® valves were installed on top of the lid for the removal of air bubbles and to pump in liquid (Fig. 1C, Fig. 2A). Polyamide (nylon) screws were used to secure the lid to the container and provide additional sealing tension (Fig. 1C, Fig. 2D). A flat removable PMMA separator was created and placed down the middle of the inside of the container for situations where two separate hemispheres are scanned simultaneously. The separator can be removed for larger samples such as whole brains.

2.2. Tissue preparation protocol

Two whole hemisphere tissue samples, one Parkinson’s disease (PD) patient and one healthy control, were obtained from the Multiple Sclerosis and Parkinson’s Tissue Bank located at Imperial College London. Ethical approval was given for the use of tissue in research projects by the Research Ethics Committee for Wales, which was additionally approved by the Maastricht University medical ethical committee. Tissue samples were stored in 10% formaldehyde in PBS at room temperature. The protocol of preparing the tissue within the container was as follows:

(1) Remove tissue from storage and wash in 3 liters of PBS for 3 x 5 min.
(2) Place tissue sample into the PMMA container.
(3) Fill container with 3 M Fluorinert™ FC-770 (perfluorocarbon).
(4) Place six open 50 ml Falcon centrifuge tubes made from polypropylene loosely on top of the tissue to keep samples fully submerged.
(5) Close container and screw shut.
(6) Further seal with Kapton® tape (DuPont™) (Fig. 2B).
(7) Position sealed container upright on custom-built metal stand in a vacuum chamber (Fig. 2E).
(8) Open valves and set vacuum to a pressure of −1 bar; leave overnight.
(9) Slowly bring pressure back to 1 bar in order to keep the tissue stable.
(10) Apply additional negative vacuum pressure in ten minute intervals until no further bubbles are seen to surface. Rotate and gently shake the container between intervals.
(11) Remove container from the chamber and fill with additional Fluorinert™ via the valves and siphon residue fixative solution.
(12) Wipe container down and tape valves into the close position.
(13) Place container within a linear low-density polyethylene (LLDPE) bag to safeguard against any possible leaks during transportation and scanning.
(14) Place bagged container within a nonferromagnetic container in the MRI scanning room (adjacent to magnet) to have the material and tissue reach temperature equilibrium.
(15) Place container within RF coil at an angle (top tilted slightly upwards) so any residual liquid can float to the top and to the edge of (or out of the readout direction of) the field of view.

The removal of air from a neuronal tissue sample prior to MR scanning is imperative as the presence of air bubbles causes artifacts (i.e., distortions) on MR images. Placing the samples in a vacuum chamber overnight at negative pressure assured that the majority of air bubbles were removed. Subsequent intervals of negative vacuum pressure and further agitation of the samples including shaking and rotating them within the container between successive vacuuming cycles benefited in the disposal of additionally trapped air. The vapor pressure of Fluorinert PC-770 is $6.568 \times 10^3$ Pa (0.056 bar) which caused minor evaporation to occur while under negative pressure that appeared like bubbles surfacing similar to air bubbles being dislodged. After multiple incidences of vacuuming and agitating the samples appeared to be free of bubbles as no further air resurfaced when placed under additional negative vacuum pressure. After the samples were placed in the scanner a preliminary multi-slice localizer was used to examine if any significant air bubbles remained within the container. If multiple or large bubbles were seen on the localizer scan the container was removed from the scanner and further negative vacuum pressure cycling was applied.

Within this set up, Kapton® tape was used for it is an unfilled thermoplastic polyimide film material with silicon adhesive used as a high-grade electrical insulator. As residue fixative solution will be freed from tissue during set up and scanning, one should be mindful of the position of the container within the MR scanner and the field-of-view (FoV) created on the MR console. Placing the container within the RF head coil at an angle where residual liquid can float to the top of the container and to the edge of (or out of the readout direction of) the FoV is advised.

2.3. MRI acquisition and data reconstruction

Samples were scanned on a 9.4T Magnetom MRI scanner (Siemens Healthcare, Erlangen, Germany) equipped with a 16 channel parallel transmit (pTx) and 31 channel receive head coil (Max Planck Institute, Tübingen, Germany) (Shajan et al., 2014). First, a localizer scan with 11 slices in all three orthogonal directions was acquired for planning. Next a 2 mm isotropic field map (Cusack and Papadakis, 2002) and dual re-focusing echo acquisition mode (DREAM) (Nehrke and Bornert, 2012) sequence were obtained to characterize B0 and B1+ map profiles, respectively. B0 and B1+ offline shimming was performed using in-house custom build MATLAB scripts (Tse et al., 2016).

The main acquisitions were performed with a custom 3D gradient echo (GRE) pulse sequence, modified to use a composite parallel excitation pulse using kT-points shimming for B1+ homogenization (Tse et al., 2016). The 3D GRE sequence was used to acquire high resolution isotropic T2* weighted data at 250 µm (FoV read; 172 mm, FoV...
Fig. 3. T2* Weighted Gradient Echo scans in ascending order of 6.98 ms, 11 ms, 16.21 ms, 20.23 ms, 24.46 ms and 30 ms showing the T2* decay of the signal. Healthy control on far left and left sides, PD patient on the right sides.

Phase; 71.2%, Repetition Time (TR); 42 ms, TE; 6.98 ms, 11.00 ms, 16.21 ms, 20.23 ms, 24.46 ms and 30.00 ms, Flip Angle (FA); 15 deg. The GRE acquisition time (TA) was 05:10:58.

Diffusion-weighted imaging (DWI) scans were obtained with the 3D kT-point diffusion weighted STimulated Echo Acquisition Mode (kT-dSTEAM) sequence (Fritz et al., 2019) along 48 directions, distributed with an electrostatic repulsion algorithm for optimal whole sphere coverage, in six groups of eight directions with a B-valve of 5009 s/mm² with four B0 volumes (at a low B-valve of 318 s/mm²). The voxel size was 1 mm isotropic with a matrix size of 144 × 180 × 116, TR 450 ms, TE 28 ms, mixing time 135 ms, and a five lines per shot segmented echo planar imaging (EPI) readout. The TA was 29:10 per volume, with diffusion-weighted volumes acquired in series of eight. Total TA for all diffusion volumes was 23:22:36. Raw data was exported offline to un-
3. Results

3.1. T2* weighted images

T2-weighted scans showed a clear visualization of the gray and white matter structures of the neuronal tissue (Fig. 3). The brain container and spacer were invisible in the MR image and the proton free liquid showed no signal. Visual representations of the GRE scans are provided in Fig. 3; six echoes at 6.98 ms, 11.00 ms, 16.21 ms, 20.23 ms, 24.46 ms and 30.00 ms are provided and show different contrasts with variable structural visibility. The echo at 16.21 ms clearly shows gray and white matter borders due to it being the closest to the T2* of these tissues. Using the six echoes a quantitative T2* (qT2*) image was computed (Fig. 4). Signal loss can be seen in the occipital lobe due a combination of low transmit B1 and low receive sensitivity, the latter resulting from that area being more distant to receiver coils.

3.2. Diffusion weighted scans

A raw B0 image and directionally-encoded color (DEC) FA map are provided in Fig. 5. The raw DWI scans had enough information to reconstruct the structural white matter connections; an overview of the entire tractogram is provided in Fig. 6. It should be noted that within the occipital lobe there was not enough signal to reconstruct fiber tracts. This was in part due to our scanning protocol having the main region of interest be the mesencephalon (basal ganglia) and motor cortex, i.e. parkinsonian regions.

4. Discussion

A dedicated container was designed for scanning postmortem brains in UHF-MR scanners. In the development process, several considerations were made to optimize the container to be easy to use within MR facilities and engender high quality images with a low number of artefacts. While postmortem imaging holds several challenges including altered T1 and T2 relaxation times as well as achieving optimized imaging contrasts at high resolution, it also grants advantages to in vivo measurements including longer scan times, less motion and physiological noise artifacts, and greater SNR.
The container here proved to have the necessary qualifications needed for postmortem imaging including ease of transportation, vacuum sealing capabilities, and compatibility with previously designed sequences and procedures. Imaging procedures using a custom GRE pulse sequence that utilize composite parallel excitation pulses with kT-points proved effective in acquiring high resolution T2* weighted data at 250 μm and DWI data at 1 mm.

Air bubbles within ventricles and cortical surfaces compromise regional information and are one of the major issues for ex vivo imaging. Likewise, as fixative solution will continuously seep from the tissue during scanning, additional measures should be made to reduce such interference. The use of Fluorinert as a proton free liquid is recommended and allows for proper removal of air bubbles within the tissue. The kinematic viscosity at 20 °C of Fluorinert (0.75 cSt) is lower than Fomblin (up to 64 cSt and higher), which causes Fomblin to move less within cortical tissue. Further improvements can be made to obtain even greater image quality including improved positioning of the tissue within the container and longer scanning times. Additionally, the feasibility of this method is not limited by MRI magnet field strength allowing images to be acquired on traditional 1.5 and 3T scanners and even stronger high-field systems (≥10T) provided the bore of the magnet affords large enough RF-coils.

In the future, the use of this container alongside modified separators can be further customized to improve positioning and submersion of tissue, hold single or multiple brain slices or separate biological tissue segmentations, and reduce bulk motion and scanner-related vibrations to increase stability while scanning. This postmortem scanning methodology has proven advantageous and can be used to improve the ability in achieving high-quality low artifact MR images of large postmortem brain tissue.

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