

## Supplemental material:

### Histology coil design:

A 3.2-inch wide copper adhesive tape (76555A726, McMaster.com, Robbinsville, NJ, USA) was used for the design of all coils considered in this study. To be perfectly parallel to each other the resulting copper strips were carefully cut and fixed on Plexiglas supports (Acrylite® FF 000-9 OP-3, Evonik Industries, Parsippany-Troy Hills, NJ, USA). Additional caution was taken during this step of the design in order to insure a homogenous RF field by obtaining both bubble-free even surfaces and an equidistant gap opening between the flat conductors. The specific height of the sample insertion gap was achieved by designing in-house low permittivity spacers using Teflon. For each coil, the spacer thickness was defined by using layers of Teflon tapes (7346A15, McMaster.com, Robbinsville, NJ, USA) (Figure 1&8). Similarly, a glass-based spacer was also fabricated in-house using layers of stacked #1 glass coverslips (individual thickness ranging from 130 µm to 170 µm) to achieve the overall desired glass height.

Prior to interfacing each coil to a dedicated turning/matching (T/M) network, all RF resonators were pre-tuned to an upper frequency using a non-magnetic fixed ceramic capacitor (ATC Corp., Huntington Station, NY, USA). The designated final Larmor operating frequency (here 301MHz) was subsequently achieved with the turning/matching (T/M) network. The homemade RF shielded T/M circuit design was based on two non-magnetic capacitive trimmers (range 0.6 to 10 pF; NMKP10HVE, Voltronics Corps., Salisbury MD, USA) (Figure 1) integrated within a dedicated holder. Ease of histology coil interchange was achieved via a BNC connector connected to a 3-cm long double-shielded coaxial cable (RG-223/U, Alpha Wire Com., Elizabeth, NJ, USA; capacitance 101-pF/meter) to minimize cable losses with capacitance seen by the T/M circuit equivalent to ~3-pF.

The short coaxial length was chosen to minimize cable losses while enabling an adequate distance from the T/M box to prevent static magnetic field perturbation around the sample. The resonance of each histology probe was fine adjusted with a Network Analyzer (VIA Echo MRI, AEA technology, Inc., Carlsbad, CA, USA) using the reflection mode (S11) (1).

### RF Coil characterization

#### Dielectric constant ( $\epsilon$ ):

The dielectric constant  $\epsilon$  was determined using a Hewlett Packard 4396A Network Analyzer equipped with a dielectric material test fixture (Hewlett Packard 16453A) based on the following equation:

$$\epsilon_d = \frac{C_d \times d_d}{A \times \epsilon_0}$$

in which  $C_d$  is the measured capacitance of the spacer at 301 MHz,  $d_d$  is the thickness of the spacer, and  $A$ , the overlapping area between the conductive plates.  $\epsilon_0$  is known as the vacuum permittivity or electrical constant ( $\approx 8.854187817620 \times 10^{-12} \text{ F}\cdot\text{m}^{-1}$ ). The calibration of the instrument was run prior to each measurement session according to the recommendations of the instrument's manufacturer using the calibration kit that includes a dielectric PTFE device of known characteristic ( $\epsilon=2.1$ ).

Several spacer slabs were produced by stacking Teflon tape (ref. #7346A15, McMaster.com, Robbinsville, NJ, USA, thickness ~50- $\mu$ m) over multiple layers compressed to form thicknesses ranging from 450- $\mu$ m (~10 layers) to 1,350- $\mu$ m (~30 layers). This resulted in a lower dielectric constant measurements ranging from 1.7 - 1.9. These values near foam Teflon characteristics ( $\epsilon$ ~1.6) (2) suggesting the likely contribution of air within the compressed layers.

When examining commercial glass slides (ref: 12-550-15, FisherBrand®) or coverslips (ref.:3312, Gold Seal® or ref.:12-548-5M, Fisherfinest® Premium), both surface-treated with a moisture resistance coating, the measurement led to  $\epsilon$ =4.7  $\pm$ 0.2 for a 1-mm thick glass slide and  $\epsilon$ =2.9  $\pm$ 0.3 for coverslips ranging from 130- $\mu$ m to 170- $\mu$ m thicknesses. Stacking 3-coverslip resulted in  $\epsilon$ =2.7 $\pm$ 0.2. Comparatively, conventional borosilicate glass widely used for laboratory glassware and standard slides has dielectric properties ranging from 4.9 to 5.3 when non-treated (3) and nearing  $\epsilon$ ~3.0-3.5 for carbon-doped (4,5) or Fluorine-doped (6,7) silica. The dielectric constants measurements of the various glass materials used in our study were reproducible with less than 10% variation showing an important difference between glass slides and coverslips (~38%). This difference may be due to the nature of the glass and coating. In contrast, we measured very little effect (less than 7%) when stacking coverslips suggesting the small contribution of the air present between glass layers.

### **RF homogeneity evaluation:**

#### ***Phantom preparation:***

The phantoms used in this study to qualitatively assess the overall RF homogeneity of each coil were based on an adhesive film for micro-plate (3501, Thermo Electron Corporation, Milford, MA, USA). While a simple square cut was sufficient for the smallest coil, larger phantoms required equally spaced punched holes to insure the even spread of the 5mM Gd-DTPA doped water throughout the surface of the RF coil coverage.

#### ***Signal intensity mapping:***

The homogeneity of the RF field for each coil was qualitatively assessed with the Gd-doped water phantom described above and using an ImageJ macro developed in-house to map color-coded contour profiles of the signal intensity. The pixel-based color map was calculated experimentally as the relative deviation of the signal intensity of a gradient echo sequence normalized to the average signal measured from an ROI at of the center of the coil or in its vicinity when not possible. Based on common practice, the uniformity of the coil (mapped in green) was defined within 10% of the signal intensity at the center of the coil (8). Signals deviating by more than 10% from the center were binned by 5% increment and mapped accordingly using the color scale depicted in figure 2.

### **Sample preparation**

#### **Fresh tissue sectioning and mounting:**

For samples obtained from freshly excised tissue, all C57 black wild type mice were sacrificed by means of an intravenous (IV) injection of sodium pentobarbital (Nembutal, 200-mg/kg) prior to transcardial perfusion with phosphate buffered saline (PBS) solution followed by buffered 4% paraformaldehyde in cold PBS to fix the tissue. Whole organs were extracted, fixed in buffered 4% paraformaldehyde for 24-hours and then placed in PBS for more than 8

weeks to minimize the variability of the physicochemical properties of the fixed tissues and the corresponding tissue relaxivities. Samples were then immersed in graded sucrose solutions for progressive dehydration over 48-hours. Cryo-sections were obtained using a LEICA CM3050S cryostat with slice thicknesses ranging from 30- $\mu\text{m}$  to 60- $\mu\text{m}$ . All slices were stored in Cryo-Protectant (9) under  $-80^{\circ}\text{C}$  for long term preservation.

Sections were brought to room temperature 2 hours prior to imaging. This time allowed for sample rehydration using a buffer solution and final sample preparation. The buffer solution used for tissue rehydration was first degassed in a 5 cm Petri dish placed in a small vacuum chamber for 30-minutes. Sectioned tissue samples were then immersed in the degassed buffer solution placed under a 1.5-Hz shaker for an additional 30 minutes. This step proved crucial to wash out the impurities within the tissue, thus minimizing the formation of micro-bubbles that can be detrimental to the MR image quality. The degassed tissue was mounted on a coverslip or glass slide and surrounded whenever space allowed with Fomblin (Solvay Solexis Inc., Thorofare, NJ, USA) as a hydrophobic interface to contain the water within the rehydrated tissue. Fomblin was also chosen for its excellent susceptibility while minimizing the MR background signal.

A second coverslip was used to sandwich and seal the tissue, thereby preventing dehydration during MR imaging. The overall resulting sample thickness was less than 400  $\mu\text{m}$  for a 100- $\mu\text{m}$  tissue section encased between two coverslips and less than 1250- $\mu\text{m}$  for a glass slide/coverslip combination. The overall glass encasing was fixed together using a small amount of super glue gel (Loctite, Henkel Consumer Adhesives Inc., Bridgewater, NJ, USA) on the 4 corners of the slides, away from the sample, to prevent perturbing the main magnetic field in the vicinity of the tissue.

Large tissue samples that covered much of the slide precluded the use of Fomblin. Instead a thick solution of DePeX mounting medium Gurr® (VWR Int'l Ltd., Ballycoolin, Dublin 15, Ireland) was spread around the extremities of the encasing as an alternative to insure effective sealing.

**Pre-mounted tissue:** Tissue sections that were already mounted within a coverslip/glass slide setup required additional steps for sample preparation. The glass slide was first removed by soaking sample in Xylene (Ref.: X3P-1GAL, Fisher Chemical, Pittsburgh, PA, USA) for three days. The resulting unprotected section remained mounted on the glass slide and was then immersed and rehydrated in degassed PBS for 1-hour before protective re-encasing with a #1 coverslip using the identical steps to the fresh tissue mounting protocol.

## **Histology**

All the histological images were acquired using a Leica BM 5000 B microscope (Buffalo Grove, IL, USA) equipped with 5 $\times$  magnification apochromatic zoom lens. The images were captured using a CX 9000 CCD digital camera (MBF, Williston, VT, USA) interfaced to a PC graphic workstation using the Picture Frame software (Optronics, Santa Barbara, CA, USA). The images were stitched together using Photoshop CS5 (Adobe, Seattle, WA, USA).

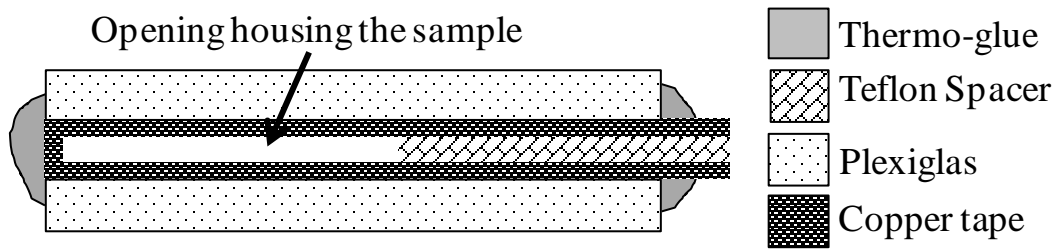
## **Data analysis:**

All the acquired images were examined and analyzed using ImageJ freeware (NIH, Bethesda, MD, USA). The SNR was calculated as the ratio between the mean signal intensity of a region of interest (ROI manually defined by the user) and the standard deviation of the background noise (ROI drawn in the corner of the image away from any ghosting artifacts).

## References

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**Figure 8 (supplemental)**



Histology coil assembly: The schematic illustrates the simplicity of our coil assembly. The rigidity of the planar copper tape conductor is insured by taping a rectangular piece of Plexiglas in each outer side of the loop with the overall structure permanently secured using thermo-glue. The thickness of the layered Teflon spacer placed between the flat copper conductors dictates the height of the opening creating a gap to insert the sample. Neither the Plexiglas nor the thermo-glue are expected to interfere with the electrical performance of the coil structure or contribute to the MR signal.

**Figure 9 (supplemental Table)**

Tissue Encasing Setup for Glass Slide and/or Coverslip	Dimension of the coils IL x W x H (mm) OL (mm)	Tissue Encasing Setup for Glass Slide and/or Coverslip	Slide Dimensions LxW (mm) Coverslip or Glass slide	Effective RF field accessible by coverslip	Sample Dimensions LxW (mm) Upper Limit (Thickness $\mu\text{m}$ )	In-Plane Resolution range ( $\mu\text{m}$ )	
						Slice thickness range ( $\mu\text{m}$ )	
						Paraffin Embedding ( $5\mu\text{m}$ - $10\mu\text{m}$ )	Cryo-section ( $30\mu\text{m}$ - $100\mu\text{m}$ )
Small RF Coverslip Coil	12.0 x 12.0 x 0.45 24.0	Dual Smallest Coverslip	24.0 x 12.0	Partial Coverage	10.0 x 10.0 (100- $\mu\text{m}$ )	40 $\mu\text{m}$ - 55 $\mu\text{m}$	15 $\mu\text{m}$ - 25 $\mu\text{m}$
Standard RF Coverslip Coil	24.0 x 26.0 x 0.45 48.0	Dual Standard Coverslip	50.0 x 24.0	Partial Coverage	20.0 x 20.0 (100- $\mu\text{m}$ )	55 $\mu\text{m}$ -75 $\mu\text{m}$	20 $\mu\text{m}$ -35 $\mu\text{m}$
Large RF Coverslip Coil	24.0 x 52.0 x 0.45 48.0			Full Coverage	43.0 x 20.0 (100- $\mu\text{m}$ )	70 $\mu\text{m}$ -95 $\mu\text{m}$	25 $\mu\text{m}$ -40 $\mu\text{m}$
Standard RF Glass Slide Coil	25.0 x 26.0 x 1.35 48.0	Standard Coverslip/Glass Slide	50.0 x 24.0 & 75.0 x 25.0	Partial Coverage	20.0 x 20.0 (150- $\mu\text{m}$ )	70 $\mu\text{m}$ -95 $\mu\text{m}$	25 $\mu\text{m}$ -40 $\mu\text{m}$
Large RF Glass Slide Coil	25.0 x 52.0 x 1.35 48.0			Full Coverage	43.0 x 20.0 (150- $\mu\text{m}$ )	95 $\mu\text{m}$ -135 $\mu\text{m}$	30 $\mu\text{m}$ -55 $\mu\text{m}$

Summary table of the dimensions of the five coils developed with corresponding commercial coverslips and glass-slide. Each coil was designed for a specific sample setup (type of coverslip or glass slide, RF coverage and sample size) while maximizing the filling factor. The resulting sensitivity of each coil is translated in this table by the in-plane resolution (the minimal in-plane pixel size requirements) in order to achieve an SNR greater than 30 within 10 hour acquisition time corresponding to an overnight unattended scan. The range of values outlined are inferred from experimental measurements obtained from mouse brain sections immersed in PBS for more than 8 weeks to minimize the variability of the physicochemical properties of the fixed tissues (insure stability of the tissue relaxivities). The flip angle in each individual MR acquisition was systematically chosen to maximize experimentally the signal intensity corresponding to the Ernst angle (best SNR conditions) (10). The in-plane resolution range was categorized based on the slice thicknesses commonly utilized in either paraffin embedding ( $5\mu\text{m}$ - $10\mu\text{m}$  sections) or cryo-sectioning ( $30\mu\text{m}$ - $100\mu\text{m}$ ). It must be noted that the outlined values were inferred from experimental measurements acquired using a 7 Tesla system for this study. In addition of the magnetic field dependence, these values can vary based on the nature and shelf-life of the tissue, the MRI sequence and tissue contrast (T1- or T2-weighting).