Quantification of Formalin-Fixed MS Brain Tissue Parameters T1, T2*, PD and Phase at 7T and Comparison with Histopathology

C. R. Zachariah[1], D. Pitt[2], P. Wassenar[1], B. D. Clymer[1], A. Abduljalil[1], M. V. Knopp[1], and P. Schmalbrock[1]
[1]Radiology, The Ohio State University, Columbus, OH, United States, [2]Neurology, The Ohio State University, Columbus, Ohio, United States

Introduction: Recent histopathologic studies have shown that multiple sclerosis not only leads to demyelination in white matter (WM), but can also lead to extensive demyelination in cortical gray matter [1-2]. It is difficult to detect cortical demyelination with conventional MR imaging techniques. Although significant improvements were achieved with ultrahigh-field MRI (7T) using 3D T1, T2* and phase methods [4-6], cortical lesion imaging still is hampered by low contrast, spatial resolution and specificity of MRI signal characteristics. MR imaging of brain tissue specimen is of interest, because it permits direct comparison with histopathology. This allows in turn for improved interpretation of the observed MRI signal characteristics. However, quantitative MR tissue parameters are known to change in formalin tissue [1]. Our goal was to measure proton density (PD), T1, T2* and phase difference in formalin fixed specimen at 7T. To our knowledge, phase differences and proton densities, or T1 and T2* at 7T for formalin fixed tissue were not yet reported.

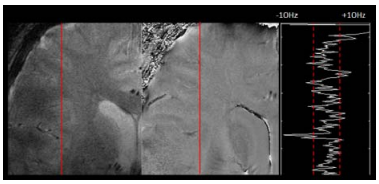

Fig 1: In vivo magnitude and ΔB_o [Hz] image of healthy volunteer. The ΔB_o [Hz] image was generated by voxel-wise linear regression of data for different TE; the graph on the right represents a ΔB_o line plot along the red line revealing GM/WM changes of about 5Hz

Methods: Human brain tissue from 4 MS patients was obtained at autopsy with IRB-approval. Brain was fixed with formalin for over 2 years. For MRI, specimen were immersed in phosphate buffered saline . Images were acquired at 7T using a transmit/receive knee coil. T1 measurements were acquired using 3D inversion recovery-turbo-field and spin echo (IR-TFE and IR-TSE) with TI=67-3500ms, and spoiled field echo (FFE) sequences with different flip angles 5-90^o (0.25x0.25x1.0mm^3 voxel size). T2* and phase images were acquired with 3D-FFE using TE=3-45ms (0.15x0.15x0.3mm^3 voxel size). T1 was computed by fitting signal in selected ROIs with the respective signal equations; T2* was computed by linear regression of logarithm of the magnitude images at different TE. PD was computed relative to PBS from the fitting scaling factors and correction T1/T2* terms.

Phase images were reconstructed from the complex raw data by high pass filtering and other methods [7]. The local magnetic field difference was computed by linear regression of the phase versus TE. Following MRI, specimen were cut and labeled with anti-myelin basic protein antibodies to detect myelin and with anti-CD68 antibodies to detect activated macrophages/microglia. Sections were also stained for iron with Perls' stain-DAB. Scanned histology slides were scored for cortical lesions and inflammatory activity within lesions and compared to MRI [8,9].

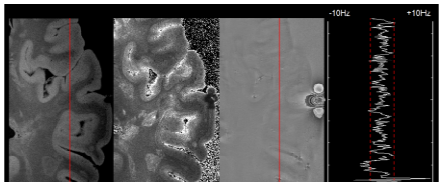

Fig 2: Specimen magnitude, T2* and ΔB_o [Hz] image and ΔB_o line plot. The GM/WM change is about 3Hz. Note that white matter lesions well seen on magnitude and T2* are barely visible on the specimen phase images. Histology of this specimen is underway

Results: Average PD, T1, T2* and local field differences for formalin fixed tissue are listed in Table 1. For comparison, also included are published in vivo data. Measurements with different methods and for different tissue specimen were fairly consistent. Due to cross-linking PD and T1 are significantly smaller. T2*-values are comparable (**Table**). We found that generation and quantification of phase images is extremely challenging. First, significant bulk magnetic field changes from air/water interfaces at the edges of the container and near unavoidable air bubbles obliterate the images, making it difficult to find suitable phase filtering or unwrapping parameters especially for TE >25ms. Further, the phase difference is smaller than in vivo (**Fig.1 and 2**). Phase contrast is inverted between axial in vivo and coronal specimen images. Note that neither magnitude nor phase specimen images depict small veins. The ring of iron containing glia is dark on magnitude and bright on phase images (**Fig 3**).

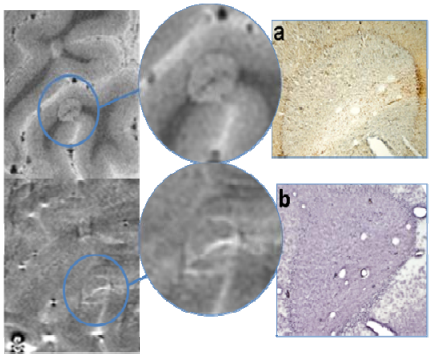

Fig 3: Specimen magnitude and phase image at TE=17ms compared to CD68(a) and Perl stain(b). Note that the ring of iron loaded glia is dark on the magnitude and bright on phase images

Discussion: The measured PD, T1 and T2* parameters will allow optimizing acquisition sequences for 7T studies of formalin fixed tissue. The data suggest that PD-weighted imaging may be promising for improving cortical lesion depiction. Our gradient echo images have mixed PD/T2*contrast explaining the improved contrast observed with this sequence, as well as the contrast observed with T2*-maps. Contrast in phase images has been attributed to venous deoxyhemoglobin, non-heme tissue iron, water protein exchange and bulk effects dependent on tissue orientation with respect to B_o [10] . As in a prior study [11], GM/WM and lesion contrast is diminished reduces on phase images. Cross-linking with formalin fixation will change water diffusion and water protein exchange. This may explain the differences observed in specimen phase images compared to in vivo studies. Further work is needed to differentiate bulk from subvoxel phase shift effects.

Table 1: Tissue Parameters at 7T

	Formalin Fixed Specimen			In vivo		
	PD	T1[ms]	T2*[ms]	PD [12]	T1 [ms] [13]	T2*[ms] [14-16]
NAWM	0.57	280	22	0.69	1220	29-36
NAcGM	0.74	350	30	0.8	2131	32
WML	0.81	500	45	-	-	-
cGML	0.83	370	40	-	-	-
PBS/CSF	≡1	>3000	>500	≡1	4425	-

References:
[1] Schmierer ISMRM09 1139,1152, 2692, [2] Geurts, J Neurol 255, 18, 2008, [3] Sati, ISMRM09, [4] Mainero, Neurology 2009, [5] Kollia, AJNR 2009, [6] Hammond, NeuroImage 39 1682, 2008, [7] Abduljalil, ISMRM09 2854, [8] Schmalbrock, ISMRM09, 3225, [9] Pitt, Arch Neurol 2010, [10] He, PNAS 106 2009 13558, [11] Yao, ISMRM09, 342, [12] Tofts, [13] Rooney MRM 57, 308, 2007 [14] Li, NeuroImage 32, 1032, 2006, [15] Yang MRM 2010 (in press), [16] Peters, MRI 25, 748, 2007