

# Myelin Water Imaging of Multiple Sclerosis at 7T: Correlations with Histopathology

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## Introduction

Various *in-vivo* MR techniques have been used to study the pathological evolution of multiple sclerosis (MS). Multi-echo T<sub>2</sub> relaxation is an MRI technique that can probe specific water environments whereby the signal from mobile protons is separated into different water pools based on their T<sub>2</sub> relaxation time. Previous *in-vivo* work at 1.5T [1-3] showed that mobile protons in healthy human brain can be separated into three compartments: (1) a very long T<sub>2</sub> component of approximately 2 seconds assigned to cerebrospinal fluid; (2) an intermediate component in the order of 80 milliseconds from intra- and extra-cellular water (IE); and (3) a short T<sub>2</sub> component of approximately 20 milliseconds arising from myelin water trapped tightly between the myelin bilayers. The latter component has been shown to be variably decreased in MS lesions [3-5], as well as diffusely reduced in the normal appearing white matter (NAWM) when compared to healthy controls [5]. Fortuitously, previous work at 1.5T has shown little change in the myelin water signal immediately after death and upon tissue fixation when compared to *in-vivo* values [6]. As well, both a good qualitative and quantitative correspondence between myelin water imaging at 1.5T and luxol fast blue (LFB) staining for myelin has been observed [7] [6]. However, a limitation of myelin water imaging at 1.5T is signal to noise. Recently, several investigators have performed myelin water imaging studies at field strengths higher than 1.5T (3T [8-10] and 7T [11]). **The goal of this study was to determine if the myelin water signal at 7 Tesla quantitatively correlated with a histopathologic marker for myelin.**

## Methods

Ten brain slices, fixed in 10% formalin, from 3 subjects with MS were examined (2 female, 1 male; mean age = 36 years (range 24-49 years); mean disease duration = 13 years (range 8-20 years)). All MRI experiments were carried out on a 7T, 30 cm bore, Bruker Advance MR scanner (Etingen, Germany). A 7 cm i.d. quadrature volume coil was used for rf pulse transmission and signal reception. Myelin water measurements were carried out using a single slice, multi-echo CPMG sequence (256x256 matrix, TR/TE=1500/6.673ms, 6 averages, 32 echoes, FOV=6 cm, slice thickness = 1 mm, in plane resolution = 234µm x 234µm). Various regions of interest (ROI's) were outlined in white matter, grey matter and lesions and the T<sub>2</sub> relaxation decay curves were decomposed into an unspecified number of exponentials by using a non-negative least squares algorithm [12]. The myelin water fraction (MWF) was defined as the fraction of the T<sub>2</sub> signal below 25ms divided by the total signal in the T<sub>2</sub> distribution. The tissue samples were then embedded in paraffin, sectioned into 10µm thick sections and stained with Luxol Fast Blue (LFB) for myelin. ROI's from the T<sub>2</sub> relaxation experiment were mapped onto the histopathology images registered to the TE=6.673ms MR image and mean optical density (OD) was determined using Image Pro Plus (Media Cybernetics, Silver Spring, MD). Correlations were investigated between LFB OD and MWF; p<0.05 was considered significant.

## Results

Figure 1 shows a T<sub>2</sub> distribution from a region of NAWM at 7T from formalin fixed brain. In comparison, T<sub>2</sub> distributions from 1.5T are also shown for NAWM in formalin and *in vivo*. Note the decreased T<sub>2</sub> time of the IE pool at 7T. Figure 2 shows two examples of excellent myelin water maps compared to the TE=20ms image. Myelin water maps showed good qualitative correlation with LFB staining, as shown in Figure 3. On average, MWF correlated strongly with LFB OD [mean (and range) R<sup>2</sup> = 0.73 (0.65-0.90), p<0.0001], an example is shown in Figure 3.

## Conclusions

1. The quality and detail of the 7T myelin water map is far superior to that previously seen at 1.5T.
2. There is excellent correspondence between the myelin water map, as generated by the short T<sub>2</sub> component, and the Luxol Fast Blue stain for myelin in formalin-fixed MS brains.
3. There is a quantitative relationship between the myelin water fraction and the intensity of Luxol Fast Blue staining of myelin [mean (range) : R<sup>2</sup>=0.73 (0.65-0.90), p<0.0001]
4. 7T imaging will allow detailed assessment of myelin pathology to a degree not possible with lower field strengths.

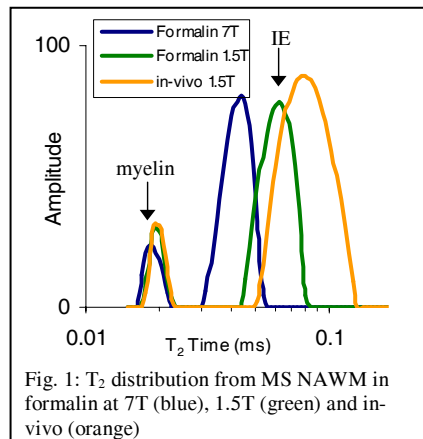


Fig. 1: T<sub>2</sub> distribution from MS NAWM in formalin at 7T (blue), 1.5T (green) and in-vivo (orange)

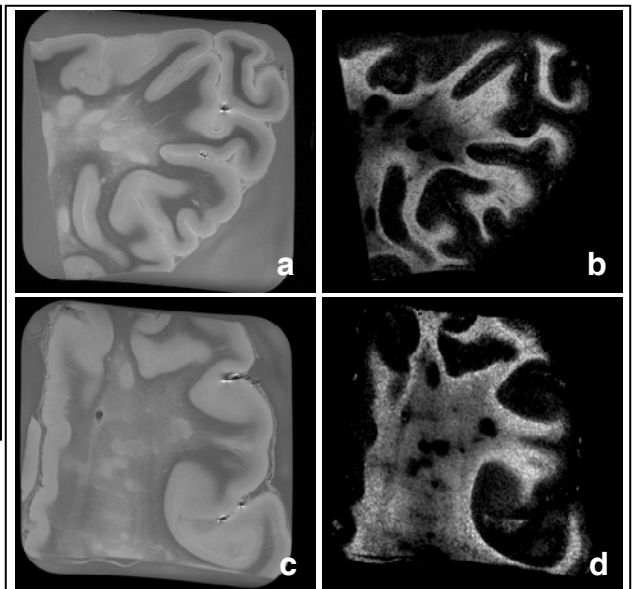


Fig. 2: Example of TE=20ms image (a,c) and myelin water map (b,d)

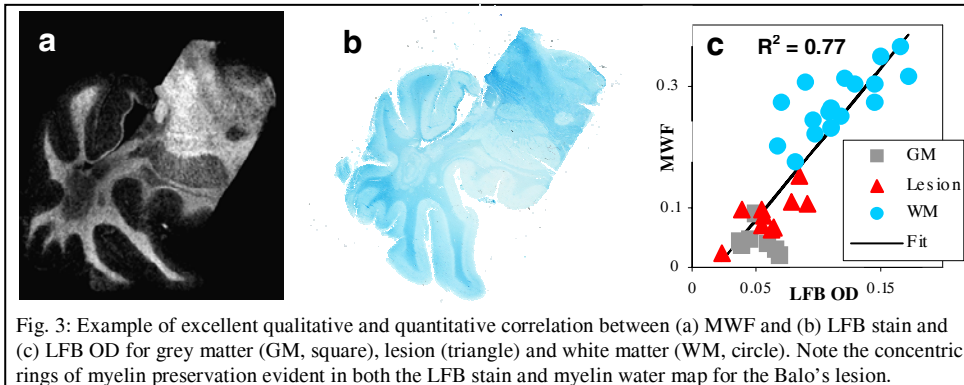


Fig. 3: Example of excellent qualitative and quantitative correlation between (a) MWF and (b) LFB stain and (c) LFB OD for grey matter (GM, square), lesion (triangle) and white matter (WM, circle). Note the concentric rings of myelin preservation evident in both the LFB stain and myelin water map for the Baló's lesion.

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