

## Myelin Water Measurement in the Presence of Myelin Debris

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### Introduction

A wide array of processes occurs after spinal cord injury, including white matter demyelination and proximal and distal Wallerian degeneration. The demyelination and interruption of the axonal tract ultimately leads to the functional loss seen in spinal cord injuries. Quantitative T2 is a technique that is used to track myelin content in spinal cords non-invasively. Because myelin cannot be imaged directly with MR due to very short T2 relaxation times, the techniques focus on indirect measurement of myelin through probing the properties of the surrounding water. It is therefore important to understand how changes in morphology affect the water environments. For this study we focused on the injured *fasciculus gracilis* 5 mm cranial to injury, where we expect, from our previous findings [1], that myelin debris are present at 3 weeks and are largely cleared by 8 weeks following injury.

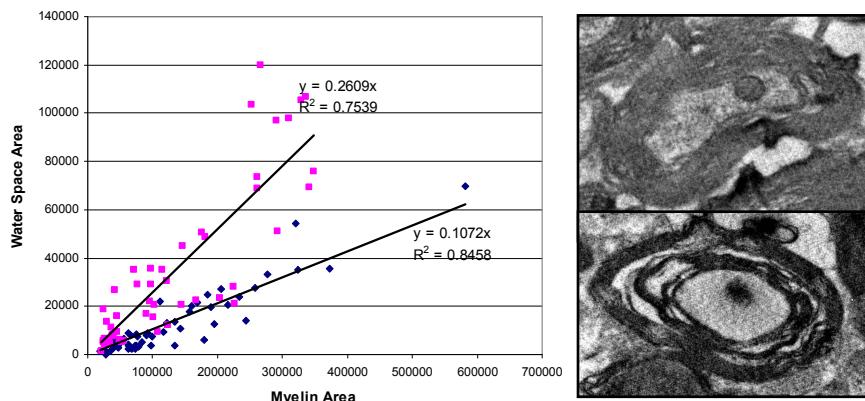
### Methods

All MRI experiments were carried out on a 7 T animal scanner (Bruker, Germany) using a 5 turn, 13 mm i.d. solenoid coil. Quantitative T2 mapping experiments were used to characterize six excised rat spinal cord samples at 3 weeks, and seven excised rat spinal cord samples at 8 weeks following dorsal column transection (DC Tx) injury [2]. A single slice multi-echo CPMG sequence was used to acquire quantitative T2 data at 5 mm cranial to injury (256 × 256 matrix, TE/TR = 1500/6.738 ms, 32 echoes, 2.56 cm FOV, 1 mm slice, NA = 6, 100  $\mu$ m in-plane resolution) [3]. CPMG data were processed using a non-negative least square analysis technique [4]. Myelin water fraction (MWF) maps were generated by dividing the integral from 7.75–20 ms range by the total integral of the T2 distribution. Region of interest analysis was used to obtain average value of MWF from the injured *fasciculus gracilis*, where myelin damage is most prominent in this model [1]. Statistical significance of difference between group mean was assessed using two tailed *t*-test. T2 relaxation times of the short and long water component were measured in the same region on a voxel-by-voxel basis. From each sample, electron micrographs (EM) were generated at 10,000 $\times$  magnification at a resolution of 2048 × 2048 pixels. These were stitch together to form a thin column. Lines were drawn at 500 pixel intervals down the column and any axon with myelin contacting the line is quantified (up to 50 intact and 50 degraded myelin areas per animal). Each individual axon and myelin sheath was manually circled. Water space area was measured as the area of water trapped within the myelin sheaths, highlighted using an intensity gradient; myelin area was measured as the area occupied by both the sheaths and the trapped water.

### Results and Discussion

Figure 1 shows an example of the result from EM analysis as well as example images of intact and damaged myelin. The plot of water space area versus myelin area reveals two distinct populations: one consisting of intact myelin and one of myelin debris. Significant difference ( $p < 0.02$ ) was found in MWF between the 3 weeks group (0.480) versus the 8 weeks group (0.318), which corresponds well with our previous results [1]. Table 1 shows the relaxation times of the short and long T2 components. Both components had shorter T2 values at 8 weeks as compared to 3 weeks post injury, although the differences were not statistically significant.

The water space area versus myelin area results suggest that there are two to three times the amount of water trapped between the sheaths in myelin debris than in intact myelin, which should lead to an increased MWF measurement. The increase spacing should also lead to longer T2 relaxation times for the myelin water component in the T2 distribution. However, with change in myelin sheath space on the order of a few hundred nanometers, the expected effect would be very small. The trend that we observed in our data for the shortening of the myelin water T2 may be due to the increased portion of myelin debris in total myelin measurement. Webb *et al.* has previously demonstrated that MWF measures both myelin and myelin debris [5]. Evidence presented here suggests that myelin debris contributes to MWF more than intact myelin. Therefore while MWF correlates well with myelin content in the absence of myelin debris, it is not a good indicator of myelin content during degeneration. In the presence of myelin debris, MWF can overestimate the amount of myelin content. This is especially critical shortly after injury where the amount of overall myelin content remains the same, while the intact myelin degenerates into myelin debris.



**Figure 1. Example water space versus myelin area measurement (left) and intact myelin (top right) and myelin debris (bottom right) from EM.** Blue denotes result from intact myelin; pink denotes result from myelin debris. Myelin debris has much larger water content than intact myelin.

**Table 1. T2 times for short and long T2 component at 3 and 8 weeks.** Changes were not statistical significant.

Time	3 weeks		8 weeks	
	T2 <sub>short</sub>	T2 <sub>long</sub>	T2 <sub>short</sub>	T2 <sub>long</sub>
Mean (ms)	13.5	32.3	12.6	30.1
S.D. (ms)	3.7	6.6	4.4	3.7

### Acknowledgement

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### References

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