

# Correcting Myelin Water Fraction for T2 Changes Caused by Varying Phosphate Buffer Concentration in Aldehyde Fixed Spinal Cord Tissue

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**Target Audience:** Our target audiences are scientists who use quantitative T2 technique in *ex vivo* studies of white matter.

## Introduction

Myelin content is an important marker for central nervous system pathology. Quantitative T2 based myelin water (MW) imaging has been shown to measure myelin content in normal and diseased brain and spinal cord tissues<sup>1,2</sup>. Because myelin is difficult to image directly with MR due to very short T2 relaxation times of myelin protons, this technique focuses on indirect measurement of myelin by probing the properties of the surrounding water. Rat models are widely used for the study of spinal cord injuries and associate repair therapies, but due to the challenges in obtaining *in vivo* images, *ex vivo* aldehyde fixed spinal cord samples are often used as an interim solution for the validation of MR techniques versus histological measurements. However, because tissue properties are altered by the fixation procedure, it is important to understand how these changes impact the measured MR parameters. It has been shown that aldehyde fixatives reduce the T2 relaxation time of brain samples<sup>3</sup> and fixation affects brain morphology overtime<sup>4</sup>. This study looks at the effect of PB concentration on the shape of T2 distributions and how MW fraction (MWF) calculations are affected.

## Methods

Six Fischer 344 rats were perfused intracardially with paraformaldehyde in 0.1 M sodium PBS for 30 minutes followed by a 3 minute long PBS solution flush. For each rat, the cervical spinal cord was then extracted and cut into four 3 mm sections. Each sample was fixed separately overnight in glutaraldehyde solutions which varied in their PB concentration (0.210 M, 0.100 M, 0.070 M, 0.046 M, 0.023 M, and 0.000 M randomly assigned).

MR experiments were carried out on a 7T animal scanner (Bruker, Germany) using a 5 turn, 13 mm i.d. solenoid coil. Quantitative T2 data were acquired using a single slice multi-echo CPMG sequence, with the slice location at C5 level (256 × 256 matrix, TE/TR = 1500/6.738 ms, 32 echoes, 1.79 cm FOV, 1 mm slice, NA = 12, 70  $\mu$ m in-plane resolution)<sup>6</sup>. CPMG data were processed using a non-negative least square analysis technique<sup>7</sup>. Geometric mean T2 (GMT2) was calculated for both the *fasciculus gracilis* and the fixative solution surrounding the sample. MWF maps were generated by integrating the range of T2 distribution associated with MW and dividing it by the total integral of the T2 distribution for each pixel. Two MW ranges were used, first one from 7.75 ms – 20 ms, corresponding to our previous studies<sup>2,5</sup>, and the second one with the upper cutoff equal to the mean T2 of the intra/extracellular (I/E) water peak and MW peak, determined from ROI analyses of the *fasciculus gracilis*.

## Results and Discussion

Figure 1 shows the dependence of GMT2 of both the fixative solution and the white matter of *fasciculus gracilis* on PB concentration. Figure 2 shows the uncorrected (MWF range 7.75 – 20 ms) and corrected (MWF range 7.75 – 13 ms) MWF. The *fasciculus gracilis* was chosen for the analyses because there is little intermingling of axons at the cervical level and it provides a consistent myelin content through all sections studied here. The results indicate a large increase in GMT2 of the fixative with increasing PB concentration. The *fasciculus gracilis* follows a similar increasing trend, albeit at a much smaller rate. This phenomenon can be explained by increase in proton exchange due to the proton-donating and proton-accepting property of the PB ion pair. There is also a curiously large initial drop in GMT2 from 0.000 M to 0.023 M with the buffered fixative solution showing greater variability at 0.000 M.

As the GMT2 shortens with decreasing concentrations of PB, both the I/E and MW water peaks are pushed to shorter T2 times and become broader, as a result, the fixed cut-off that worked well at our standard concentration of 0.21 M starts to classify part of the I/E peak as MW, artificially inflating the reported MWF. To correct for this effect, MWF was generated with the upper cutoff reduced to the mean T2 of I/E and MW water peak of the *fasciculus gracilis*. With this corrective step in place, the MWF reading appears to stabilize to “historical” levels with the exception in the absence of PB, where the MW peak have been reduced to less than twice the echo spacing and can no longer be reliably quantified.

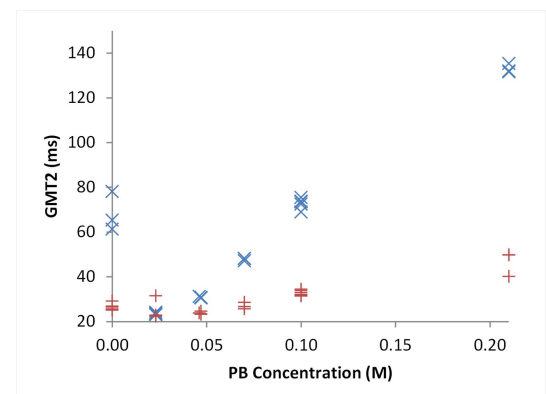
## Conclusion

With so many factors affecting the measured value of MWF, investigators need to be cautious with the details of the fixation protocol when comparing *ex vivo* results and extrapolating them to *in vivo* results.

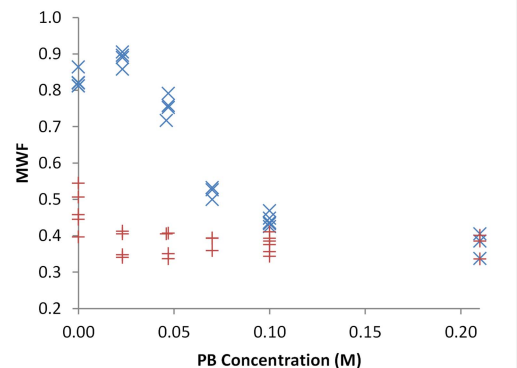
## Acknowledgment

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**References:** 1. Chan *et al.*, Exp Neurol **196**, 352 (2005); 2. Kozlowski *et al.*, Magn Reson Med, **59**, 796 (2008); 3. Shepherd *et al.*, Magn Reson Med. 2009 July; **62**, 26 (2009); 4. Guzman *et al.*, Proc. Intl Soc Mag Reson Med, **21**, 271 (2013); 5. Chen *et al.*, Proc Intl Soc Mag Reson Med, **21**, 1114 (2013); 6. Poon CS, *et al.* J Magn Reson Imaging, **2**, 541 (1992); 7. Whittall *et al.*, Magn Reson Med, **84**, 134 (1997).



**Figure 1. Dependence of geometric mean T2 (GMT2) on phosphate-buffer concentration.** The measurement of GMT2 in both the *fasciculus gracilis* (red +) and the fixative solution (blue x) indicate an increasing trend above 0 M of phosphate-buffer concentration.



**Figure 2. Dependence of myelin water fraction on phosphate-buffer concentration.** Uncorrected MWF (blue x) show decreasing trend with buffer concentration as previously reported; however, when the MW range is corrected for the changing T2 of the fixative this trend disappears.