VALIDATION AND OPTIMIZATION OF MYELIN WATER IMAGING IN A
PRECLINICAL MODEL OF SPINAL CORD INJURY

by

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Abstract

Myelin content is an important marker for neuropathology; however, direct imaging of myelin is difficult. Consequently, quantitative T2 based myelin water imaging measures myelin content indirectly by probing the property of the surrounding water. Typically, a lengthy multi-echo spin-echo sequence is used to obtain decay curves that are fitted to produce T2 distributions. In white matter, two peaks are observed, one with short and one with long T2 associated with water trapped between the myelin lipid bilayers and intra/extracellular water. The ratio of myelin water peak to the entire distribution is called the myelin water fraction (MWF) and correlates well the myelin content.

This thesis has two parts. The first half deals with the use of compressed sensing (CS) to accelerate the lengthy sequence used in myelin water imaging. The CS CPMG sequence was implemented in 2D utilizing group-sparse reconstruction in order to take advantage of the correlation between echoes. Simulated undersampling and real undersampling experiments were performed. It was found that acceleration up to 2× was possible without impacting MWF map quality, wherever adequate SNR was available. This is followed by a brief investigation into 3D CS CPMG, where similar results were achieved.

The second part of the thesis focuses on myelin water imaging in the presence of myelin debris. Because MWF is associated with the water trapped in between the myelin lipid bilayers, the reading depends heavily on myelin morphology. I compared MWF to transmission electron microscopy (TEM) derived myelin fraction using a rat injury model at normal (normal myelin), 3
weeks post-injury (a large amount of myelin debris), and 8 weeks post-injury (myelin debris partially cleared). I found that myelin water fraction correlated strongly with the amount of myelin lipid bilayers in both intact myelin and myelin debris. From the TEM images, it appears that myelin debris consists of areas of either normally spaced myelin or large watery spaces. No significant difference was found in myelin period among the three groups.
Preface

All procedures involving animals in this project were performed in accordance with the Canadian Council on Animal Care and approved by the UBC Animal Care Committee under Protocol #A12-0044.

The idea of studying myelin debris in MR using a rat spinal cord injury model was suggested by Dr. Piotr Kozlowski. The studies concerning myelin debris are designed in conjunction with him. Dr. Angshul Majumdar approached us for application for his group-sparse compressed sensing reconstruction. Of several suitable applications, myelin water imaging was one of them and Chapter 2 was born out of this collaboration.

A portion of Chapter 2 has been published in (1), which in turn was based on a conference abstract (2). I performed the sequence programming and all the experiments. I also wrote the majority of the manuscript, except for section 2.1.1, which was drafted by Dr. Angshul Majumdar. Dr. Angshul Majumdar provided the original group-sparse compressed sensing reconstruction code in Matlab, which I modified for my specific application of myelin water imaging. Section 2.5 was based on another conference abstract (3) that extended the idea further.

Injury model and transmission electron microscopy work were done in collaboration with Dr. Wolfram Tetzlaff’s laboratory at ICORD. Dr. Jie Liu performed the dorsal column transection injuries. Nathan Holmes was responsible for histology until his unexpected departure from the projects due to illness. Therefore, his contribution was limited to performing sample fixations in
work presented in Chapter 3 through 6, and electron microscopy imaging in Chapter 4 and 5. I performed the rest of electron microscopy work, including sample preparation, after receiving electron microscopy training from Susan Shinn. All of the MR experiments and data analysis were performed by me.

Chapter 4 is based on a pair of abstract (4,5) presented at ISMRM annual meetings. Chapter 5 (6,7) follows the same structure. Finally, Chapter 6 has been presented at the 24th ISMRM meeting (8) and a manuscript is being prepared for publication, which the chapter is based on.

The transceiver solenoid coils used for data acquisition are designed and built by Andrew Yung.

The project is funded by NSERC (RGPIN-2014-04849).
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<th>Description</th>
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<tbody>
<tr>
<td>$D_{\text{long}}$</td>
<td>longitudinal diffusivity</td>
</tr>
<tr>
<td>$D_{\text{trans}}$</td>
<td>transverse diffusivity</td>
</tr>
<tr>
<td>$T1$</td>
<td>spin-lattice relaxation</td>
</tr>
<tr>
<td>$T2$</td>
<td>spin-spin relaxation</td>
</tr>
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</table>
**List of Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ADC</td>
<td>apparent diffusion coefficient</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill</td>
</tr>
<tr>
<td>CS</td>
<td>compressed sensing</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>DC</td>
<td>dorsal column</td>
</tr>
<tr>
<td>DTI</td>
<td>diffusion tensor imaging</td>
</tr>
<tr>
<td>FA</td>
<td>fractional anisotropy</td>
</tr>
<tr>
<td>FID</td>
<td>free induction decay</td>
</tr>
<tr>
<td>FOV</td>
<td>field of view</td>
</tr>
<tr>
<td>GM</td>
<td>gray matter</td>
</tr>
<tr>
<td>GMT2</td>
<td>geometric mean T2</td>
</tr>
<tr>
<td>GRASE</td>
<td>gradient- and spin-echo</td>
</tr>
<tr>
<td>I/E</td>
<td>intra/extracellular</td>
</tr>
<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MT</td>
<td>magnetization transfer</td>
</tr>
<tr>
<td>MW</td>
<td>myelin water</td>
</tr>
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xvi
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>MWF</td>
<td>myelin water fraction</td>
</tr>
<tr>
<td>MWI</td>
<td>myelin water imaging</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NMSE</td>
<td>normalized mean square error</td>
</tr>
<tr>
<td>NNLS</td>
<td>non-negative least squares</td>
</tr>
<tr>
<td>OsFeCN</td>
<td>osmium tetroxide-potassium ferrocyanide</td>
</tr>
<tr>
<td>PB</td>
<td>phosphate buffer</td>
</tr>
<tr>
<td>PE</td>
<td>phase encode</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>SCI</td>
<td>spinal cord injury</td>
</tr>
<tr>
<td>SE</td>
<td>spin-echo</td>
</tr>
<tr>
<td>SNR</td>
<td>signal to noise ratio</td>
</tr>
<tr>
<td>SPGL1</td>
<td>Spectral Projected Gradient for L1 minimization</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>Tx</td>
<td>transection</td>
</tr>
<tr>
<td>WM</td>
<td>white matter</td>
</tr>
<tr>
<td>ZF</td>
<td>zero filled</td>
</tr>
</tbody>
</table>
Acknowledgements

I would first like to express my sincerest gratitude to my supervisor, Piotr Kozlowski, without whom none of this would be possible (and of course, for putting up with me). Thank you for your guidance, support, and patience throughout this journey. It has been a privilege.

I would also like to thank my committee members, Dr. Alex MacKay, Dr. Wolfram Tetzlaff, and Dr. Stefan Reinsberg, for all the helpful suggestions and discussion. Your advice and feedback had been invaluable over the past few years.

I would like to thank Andrew Yung for his technical assistance in scanner hardware and operation. This manuscript would not have existed without his mad coil building skills.

Acknowledgment and thanks go to Dr. Jie Lu, for his supreme skills in microsurgery. I think he enjoyed the field trips from ICORD to our lab, as much as we enjoyed having him over.

Big thanks go to Susan Shinn at ICORD, who trained me in the use of all things electron microscopy after the sudden departure of a collaborator that left me scrambling. I think I have stolen more than my fair share of chocolate covered coffee beans from her office. Speaking of coffee…

Some believe that science runs on coffee, so a special shout out to Barry Bohnet, who has made coffee freely available at the 7T lab since 2011. I merely piggybacked on his addiction. And to
everyone who has come through the 7T lab, past and present, you are what made it awesome, and I am glad to have been a part of your lives.

As always, special thanks are owed to my parents, who have supported me throughout my (far too) many years of education, and my brother Jack, who is an endless source of fist bumps among everything else (because awesome, and he knows).

Lastly, I would like to acknowledge Natural Sciences and Engineering Research Council of Canada for funding this project.

Thanks, everyone! It has indeed, been interesting times.
Dedication

To science. I hope I’m not ruining it.
Chapter 1: Introduction

This thesis is divided into three parts: the first part deals with improving the speed of myelin water imaging (MWI) using compressed sensing, the second part deals with the effect of fixation on MWI, and the last part deals with MWI in the presence of myelin debris. The main body of work is prefaced by the basis of magnetic resonance imaging and a brief background on the relevant anatomy and biology.

1.1 Magnetic Resonance Imaging

Magnetic resonance (MR) imaging is a medical imaging technique capable of providing anatomical, physiological, functional, chemical, and molecular information. The first MR image was obtained by Lauterbur in 1973 (9), with the first clinical images following in the early 1980’s.

While many would argue that MR is ultimately a quantum mechanical phenomenon, it happens at a sufficiently large scale that it can be understood from a classical perspective (10). Therefore, this thesis will present a classical description of MR after showing that the quantum mechanics involved readily reduces to classical mechanics. The following sections are largely derived from Haacke et al. (11) with figures based on Hanson (10).

1.1.1 Basics of Nuclear Magnetic Resonance

The basis of MR imaging is the phenomenon of nuclear magnetic resonance (NMR), where nuclei with non-zero spin in a magnetic field absorb and emit electromagnetic radiation at a
specific frequency depending on the strength of the applied magnetic field and the property of the nuclei. This stems from the fact that any nucleus with an odd number of protons and/or neutrons will have a non-zero spin, resulting in possession of a magnetic moment. The magnetic moment causes it to precess at a specific frequency when placed in a magnetic field. In MR imaging, $^1$H is of primary interest due to its natural abundance and convenience of T1 and T2 values.

<table>
<thead>
<tr>
<th>Element</th>
<th>Isotopes</th>
<th>Nuclear Spin</th>
<th>$\gamma / 2\pi$ (MHz/T)</th>
<th>Natural Abundance of Isotope (%)</th>
<th>Biological Abundance of Element (%)</th>
<th>Biological Abundance of Isotope (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>$^1$H</td>
<td>1/2</td>
<td>42.576</td>
<td>99.985</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>Oxygen</td>
<td>$^{17}$O</td>
<td>5/2</td>
<td>-5.7716</td>
<td>0.038</td>
<td>26</td>
<td>0.0099</td>
</tr>
<tr>
<td>Carbon</td>
<td>$^{13}$C</td>
<td>1/2</td>
<td>10.705</td>
<td>1.11</td>
<td>9.4</td>
<td>0.10</td>
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<tr>
<td>Phosphorus</td>
<td>$^{31}$P</td>
<td>1/2</td>
<td>17.235</td>
<td>100</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>Sodium</td>
<td>$^{23}$Na</td>
<td>3/2</td>
<td>11.262</td>
<td>100</td>
<td>0.041</td>
<td>0.041</td>
</tr>
</tbody>
</table>

*Table 1.1. Abundance and selected NMR properties of various isotopes.* Selected NMR properties of various stable isotopes with half-integer spin are shown along with their biological abundance. Hydrogen has a biological abundance of 63%, and because 99.9985% of all hydrogen is in the form of $^1$H, the overall biological abundance of $^1$H is approximately 63%. Table adapted from Handbook of Chemistry and Physics, 53 ed., ed. by D.R. Lide, Chemical Rubber Company, Cleveland, Ohio, 1998 and Magnetic Resonance in Medicine and Biology by M.A. Foster. Pergamon Press, New York, 1984 (12,13).
1.1.2 The Quantum Preamble

Consider a stationary proton having wave function $\Psi(\vec{r},t)$ that satisfies the time-dependent Schrödinger equation,

$$i\hbar \frac{\partial \Psi(\vec{r},t)}{\partial t} = \hat{H}\Psi(\vec{r},t),$$

(1.1)

where $\hbar$ is the reduced Planck’s constant, $t$ is the time, $\vec{r}$ a position vector in space, and $\hat{H}$ is the Hamiltonian operator, representing the total observable energy of the system. If the Hamiltonian is explicitly time-independent, $\Psi(\vec{r},t)$ can be written as product of two functions such that,

$$\Psi(\vec{r},t) = \psi(\vec{r})\phi(t).$$

(1.2)

Substituting Eq. (1.2) into Eq. (1.1) and rearranging gives

$$i\hbar \frac{\partial \phi(t)}{\partial t} = \frac{\hat{H}(\psi(\vec{r}))}{\psi(\vec{r})} \phi(t).$$

(1.3)

The two sides of the equation are equal if and only if they are equal to the same constant. This finding is true because the left side is strictly time dependent while the right side is only spatially dependent. Setting this constant to be the energy, $E$, results in two separate equations, the time-independent Schrödinger equation

$$\hat{H}(\psi(\vec{r})) = E\psi(\vec{r}),$$

(1.4)

and the time-dependent differential equation

$$i\hbar \frac{\partial \phi(t)}{\partial t} = E\phi(t),$$

(1.5)

which has the solution
\[ \phi(t) = Ce^{-iEt/\hbar}, \]

where \( C \) is an arbitrary constant. For a particle in a potential field, the Hamiltonian has the familiar form of kinetic energy plus potential energy:

\[
\hat{H} = \frac{1}{2m} \hat{\mathbf{\dot{R}}}^2 + U(\hat{\mathbf{R}}) = \frac{1}{2m} \hat{\mathbf{\dot{P}}}^2 + U(\hat{\mathbf{R}}),
\]

where \( \hat{\mathbf{R}} \), \( \hat{\mathbf{V}} \), and \( \hat{\mathbf{P}} \) are the position, velocity, and momentum operators, respectively. Now, consider a stationary proton subjected to an external magnetic field pointing in the \( z \)-direction with magnitude \( B_o \):

\[ \hat{\mathbf{B}} = B_o \hat{\mathbf{z}}. \]

The potential field then becomes

\[ U(\hat{\mathbf{R}}) = -\hat{\mathbf{\mu}} \cdot \hat{\mathbf{B}}, \]

where \( \hat{\mathbf{\mu}} \) is the magnetic moment operator of the proton and is related to the total angular momentum operator, \( \hat{\mathbf{J}} \), by the gyromagnetic ratio, \( \gamma \), of the proton such that

\[ \hat{\mathbf{\mu}} = \gamma \hat{\mathbf{J}}. \]

Note that

\[ \hat{\mathbf{J}} = \hat{\mathbf{S}} + \hat{\mathbf{L}}, \]

where \( \hat{\mathbf{S}} \) is the spin angular momentum operator and \( \hat{\mathbf{L}} \) the orbital angular momentum operator. The orbital angular momentum operator is analogous to the classical definition of
angular momentum. Conveniently, because $^1\text{H}$ has only one electron in the s shell, which is spherically symmetrical, it has no orbital angular momentum. Therefore, its total angular momentum is that of a proton, so

$$\hat{\mu} = \gamma \hat{S},$$

and the potential field can be rewritten as

$$U(\hat{R}) = -\gamma B_o \hat{S}_z,$$

where $\hat{S}_z$ is the operator form of the spin angular momentum in the $z$-direction. Assume the proton is stationary and therefore has zero momentum, the Hamiltonian becomes

$$\hat{H} = -\gamma B_o \hat{S}_z.$$  

The spin angular momentum operator can be described by Pauli spin matrices:

$$\hat{S}_z = \frac{\hbar}{2} \sigma_z = \frac{\hbar}{2} \begin{bmatrix} 1 & 0 \\ 0 & -1 \end{bmatrix},$$

so

$$\hat{H} = -\frac{\gamma B_o \hbar}{2} \begin{bmatrix} 1 & 0 \\ 0 & -1 \end{bmatrix}.$$  

Using the Larmor precession frequency equation,

$$\omega_o = \gamma B_o,$$

gives a more familiar version Eq. (1.16):
\[ \hat{H} = \begin{bmatrix} -\frac{1}{2} \hbar \omega_o & 0 \\ 0 & \frac{1}{2} \hbar \omega_o \end{bmatrix}. \]  

(1.18)

To satisfy the time-independent Schrödinger equation, the complete orthonormal basis set of eigenstates must be

\[ \phi_\uparrow = \begin{bmatrix} 1 \\ 0 \end{bmatrix}, \]

(1.19)

and

\[ \phi_\downarrow = \begin{bmatrix} 0 \\ 1 \end{bmatrix}. \]

(1.20)

By substituting Eqs (1.18-20) back into the time-independent Schrödinger equation, the energy of the eigenstates can be found. The energy eigenvalue related to each eigenstate is then

\[ E_\uparrow = -\frac{1}{2} \hbar \omega_o, \]

(1.21)

and

\[ E_\downarrow = \frac{1}{2} \hbar \omega_o. \]

(1.22)

This is called Zeeman splitting, with \( E_\uparrow \) corresponding to the lower energy spin-up state that aligns with \( B_o \) and \( E_\downarrow \) corresponding to the higher energy spin-down state aligned antiparallel with \( B_o \). (Note that MR measurement never forces any individual proton spin into one of the two eigenstates). Recall from Eq. (1.2) that \( \Psi(\vec{r},t) \) can be constructed from the time-dependent portion of the two eigenstates \( \phi_\uparrow \) and \( \phi_\downarrow \) with probability \( C_\uparrow \) and \( C_\downarrow \), respectively:
\[ \Psi(\vec{r}, t) = C_1 \psi_1 e^{-iE_1 t/\hbar} + C_2 \psi_2 e^{-iE_2 t/\hbar}. \]  

Substituting the energy eigenvalues from the Hamiltonian [Eqs (1.13) and (1.14)] into Eq (1.23), the general state wave function becomes

\[ \Psi(t) = C_1 \psi_1 e^{-i\omega t/2} + C_2 \psi_2 e^{-i\omega t/2}. \]  

In Dirac bra-ket notation, the ket is then

\[ |\Psi(t)\rangle = C_1 e^{i\omega t/2} \begin{bmatrix} 1 \\ 0 \end{bmatrix} + C_2 e^{-i\omega t/2} \begin{bmatrix} 0 \\ 1 \end{bmatrix} = \begin{bmatrix} C_1 e^{i\omega t/2} \\ C_2 e^{-i\omega t/2} \end{bmatrix}, \]  

and its Hermitian conjugate, bra, is

\[ \langle \Psi(t)| = C_1^\dagger e^{-i\omega t/2} \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} + C_2^\dagger e^{i\omega t/2} \begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix} = \begin{bmatrix} C_1^\dagger e^{-i\omega t/2} & C_2^\dagger e^{i\omega t/2} \\ C_2^\dagger e^{-i\omega t/2} & C_1^\dagger e^{i\omega t/2} \end{bmatrix}. \]  

The normalization for this system is

\[ \langle \Psi(t)|\Psi(t)\rangle = 1, \]  

which means that

\[ |C_1|^2 + |C_2|^2 = 1. \]  

Assuming that the probability densities \( C_1 \) and \( C_2 \) are of the form

\[ C_1 = U e^{i\nu}, \]  

and

\[ C_2 = D e^{i\delta}, \]  

where \( U \) and \( D \) are amplitudes and \( \nu \) and \( \delta \) are arbitrary phases for the spin-up and spin-down states, Eq. (1.28) reduces to
\[ U^2 + D^2 = 1. \]  

To account for all initial conditions, an arbitrary angle \( \Theta \) can be introduced so that

\[ U = \cos \Theta, \]  

and

\[ D = \sin \Theta. \]  

Putting it all together, the system \textit{bra} is

\[
\ket{\Psi(t)} = \begin{bmatrix} \cos \Theta e^{i(\alpha t/2 + \delta)} \\ \sin \Theta e^{-i(\alpha t/2 - \delta)} \end{bmatrix}.
\]  

(1.34)

And the system \textit{ket} is

\[
\bra{\Psi(t)} = \begin{bmatrix} \cos \Theta e^{-i(\alpha t/2 + \delta)} & \sin \Theta e^{i(\alpha t/2 - \delta)} \end{bmatrix}.
\]  

(1.35)

From Eqs (1.34) and (1.35) the expected value of the magnetic moment can be calculated by,

\[
\langle \hat{\mu} \rangle \equiv \langle \Psi(t) | \hat{\mu} | \Psi(t) \rangle = \frac{\gamma \hbar}{2} \langle \Psi(t) | \hat{\sigma} | \Psi(t) \rangle.
\]  

(1.36)

Breaking this down into its respective components yields:

\[
\langle \hat{\mu}_x \rangle = \frac{\gamma \hbar}{2} \langle \Psi(t) | \hat{\sigma}_x | \Psi(t) \rangle,
\]  

(1.37)

\[
\langle \hat{\mu}_y \rangle = \frac{\gamma \hbar}{2} \langle \Psi(t) | \hat{\sigma}_y | \Psi(t) \rangle,
\]  

(1.38)

and

\[
\langle \hat{\mu}_z \rangle = \frac{\gamma \hbar}{2} \langle \Psi(t) | \hat{\sigma}_z | \Psi(t) \rangle.
\]  

(1.39)
Let $\varphi_0 = \delta - \nu$ (initial phase difference) and $\theta = 2\Theta$, then

$$
\langle \hat{\mu}_x \rangle = \frac{\gamma h}{2} \sin \theta \cos (\varphi_0 - \omega_0 t),
$$

(1.40)

and

$$
\langle \hat{\mu}_z \rangle = \frac{\gamma h}{2} \sin \theta \sin (\varphi_0 - \omega_0 t),
$$

(1.41)

These equations represent a vector with a magnitude of $\gamma h/2$ precessing at the Larmor frequency, at an angle of $\theta$ about the $z$-axis. Thus the quantum mechanical derivation for a spin $\frac{1}{2}$ particle in a constant external magnetic field reduces to the classical prediction that will be presented in the next section. Note that even though the derivations so far applies to a single proton and not an ensemble, the result is the same for macroscopic magnetization (14).

### 1.1.3 The Semi-Classical Approach

As described previously, NMR results from the interaction of the permanent magnetic moment, $\hat{\mu}$, of an atomic nucleus with an external magnetic field. When a spin is placed in an external magnetic field, $\vec{B}$, it experiences a torque, given by

$$
\vec{\Gamma} = \vec{\mu} \times \vec{B}.
$$

(1.43)

The torque exerted produces a change in angular momentum, $\vec{J}$ according to

$$
\vec{\Gamma} = \vec{\mu} \times \vec{B} = \frac{\partial \vec{J}}{\partial t}.
$$

(1.44)
This causes the nuclei to precess at an angle about the external field at the Larmor precessional frequency defined by Eq. (1.17).

![Diagram of MR coordinates]

**Figure 1.1. Conventional MR coordinates.** In a typical MR scanner, the coordinate system is right handed with the external magnetic field $B_0$ pointed along the bore of the magnet. Depending on the scanner manufacturer, $y$ and $x$ may be defined differently but $z$ always points along $B_0$.

If $\vec{B}$ is in the $z$ direction (by NMR convention, the external field lies along the $z$-axis, which is referred to as the longitudinal axis; the $x$- and $y$-axes form the transverse plane, see Figure 1.1), Using this coordinate system, we can write

$$J_z = m_s \hbar,$$

and the $z$-component of the magnetic moment orients according to:

$$\mu_z = m_s \gamma \hbar,$$

where the magnetic quantum number, $m_s$, can be any value from $-I$ to $+I$ in integer steps. The direct result of this is a splitting of energy level. For spin $1/2$ nuclei ($e.g.$, a proton),

$$|\mu_z| = \frac{\gamma \hbar}{2}.$$
Note that this equation is the same as the result obtained from the quantum mechanical analysis. Two energy levels exist for spin-$\frac{1}{2}$ nuclei, corresponding to spins oriented parallel or antiparallel with respect to the applied magnetic field. (Now if we follow through with the x and y components, we will get the behaviour of the precession that matches Eqs. 1.40 and 1.41). The energies of these levels are given by the classical formula for a magnetic dipole in a homogeneous magnetic field of strength $B_0$:

$$E = -\mu_z B_0.$$  

(1.48)

Hence,

$$E = -m_s \gamma \hbar B_0,$$  

(1.49)

and the energy difference between the two spin states of spin-$\frac{1}{2}$ nuclei is

$$\Delta E = \hbar \gamma B_0.$$  

(1.50)

The transition from the lower energy state to the upper energy state (i.e., resonance absorption) happens when electromagnetic radiation of the correct frequency to match this energy is applied. Therefore,

$$\hbar \omega_o = \Delta E = \hbar \gamma B_0,$$  

(1.51)

and so the absorption happens at the Larmor frequency. For MR imaging, the frequency usually falls in the radio frequency (RF) range. At a magnetic field strength of 7 T, $\omega_o$ is approximately 300 MHz for proton. In magnetic resonance imaging, hydrogen atoms in water molecules are of primary interest, which gives $\gamma = 42.58$ MHz / T and spin of $\frac{1}{2}$. The natural and biological abundance, as well as the high gyromagnetic ratio of the hydrogen present in the water molecule combines to yield a large signal relative to other isotopes (12,13). These characteristics,
combined with the convenience of the range of $T_1$ and $T_2$ value of the hydrogen proton, make it the dominant nucleus in MR.

Zeeman splitting occurs because of the two possible basis states of different energy. For a large collection of spins in equilibrium at a given temperature where quantum effects are negligible, the ratio of spins in the upper energy state, $N^-$, to those in the lower energy state, $N^+$, is governed by Maxwell-Boltzmann statistics,

$$\frac{N^-}{N^+} = e^{-E/kT} ,$$ (1.52)

where $k$ is the Boltzmann constant ($1.3806503 \times 10^{-23}$ m$^2$ kg s$^{-2}$ K$^{-1}$) and $T$ is the temperature.

The spin excess is typically only a few parts per million, but this excess is sufficient to provide a measurable net magnetization in the $z$-direction,

$$\overline{M} = \sum \mu ,$$ (1.53)

which is the source of the NMR signal.

Now, before discussing RF pulses, it is useful to define a frame of reference, with coordinates $x'$, $y'$, and $z'$, which rotates at the Larmor frequency about the positive $z$-axis, clockwise. Using this rotating reference frame, nuclei precessing at the Larmor frequency would appear stationary (as soon as the protons are placed into the magnet they start precessing); those precessing at higher frequencies rotate clockwise about the $z$-axis and those precessing at slower frequencies rotate counter-clockwise, i.e. slower precessing nuclei would rotate at a positive frequency relative to the rotational frame and faster-precessing nuclei at negative frequency. Consider a magnetic field
in the $x'y'$ plane that rotates around the $z$ axis with the Larmor frequency. This field is called the transmit RF field, $\vec{B}_1$, and is generated by the RF transmitter coil. $\vec{B}_1$ causes $\vec{M}$ to rotate about the direction of the transmit RF field. In NMR (and MRI), the $B_1$ field is typically applied in the form of short pulses. Depending on the duration, $\tau$, and the magnitude of $\vec{B}_1$, the rotation angle, or flip angle, $\Delta \theta$, of the magnetization is determined by

$$\Delta \theta = \int_0^\tau \gamma B_1(t)$$

(1.54)

In NMR notation, a $90^\circ_{x'}$ pulse is one which rotates $\vec{M}$ clockwise by $90^\circ$ about the $x'$ axis down to the $y'$ axis. Consequently, $180^\circ_{y'}$ pulse rotates $\vec{M}$ about $y'$ down to the $-z$ axis, assuming that $\vec{M}$ is at equilibrium (Figure 1.2).

![Figure 1.2](image)

**Figure 1.2.** a) $90^\circ_{x'}$ pulse and b) $180^\circ_{y'}$ in the rotating frame of reference. The $90^\circ_{x'}$ pulse rotates $M_o$ clockwise by $90^\circ$ about the $x'$ axis down to the $y'$ axis. The $180^\circ_{y'}$ pulse rotates $M_o$ about $y'$ down to the $-z$ axis.
1.1.4 T1/Spin-lattice Relaxation

By exciting the nuclear spins with RF energy equal to the energy difference between the spin states, it is possible to nutate a portion of the longitudinal magnetization, $M_z$, from its equilibrium value ($M_o$) into the transverse plane. The return of $M_z$ to equilibrium after the application of an RF pulse is governed by the spin-lattice relaxation time constant, T1. It implies that the rate of change of the longitudinal magnetization is proportional to the difference between $M_o$ and $M_z$:

$$\frac{dM_z}{dt} = \frac{1}{T1}(M_o - M_z)$$  \hfill (1.55)

The general solution of Eq. (1.55) is

$$M_z(t) = M_z(t_o) e^{-t-t_o)/T1} + M_o \left(1 - e^{-t-t_o)/T1}\right).$$  \hfill (1.56)

For example, if enough energy is applied, $M_z$ can be set to zero, which is the case in a 90° RF pulse. In this case, the return of $M_z$ to equilibrium follows the equation

$$M_z(t) = M_o \left(1 - e^{-t/T1}\right).$$  \hfill (1.57)

A complete inversion of the equilibrium magnetization can also be achieved, as is the case in an 180° RF pulse, in which case the return to equilibrium magnetization is

$$M_z(t) = M_o \left(1 - 2e^{-t/T1}\right).$$  \hfill (1.58)

In practice, this equation is more complicated because of imperfect inversion. When this happens we no longer have $-M_o$ at $t = 0$, so Eq. (1.56) has to be used.

1.1.5 T2/Spin-Spin Relaxation

Spin-spin relaxation causes the exponential decay of the transverse magnetization, $M_\perp$. It is a phenomenon where the net magnetization starts to dephase irreversibly, due to dipole-dipole
interaction, spin diffusion, cross relaxation and other processes. These effects cause spins to precess at a different Larmor frequency, and thereby gradually dephase magnetization over time, until \( M_\perp \) decays back to zero, causing an apparent loss of NMR signal. Spin-spin relaxation is characterized by the equation

\[
M_\perp (t) = M_\perp (0) e^{-t/T_2},
\]

that describes the decay of transverse magnetization.

Several factors also govern T2, including molecular interactions and effects due to diffusion. Fluctuating fields, which perturb the energy levels of the spin states, also cause the transverse magnetization to dephase. Ideally, there should be no spin dephasing from a homogeneous applied field; practically, however, there is an additional, recoverable, dephasing due the inhomogeneity in the applied magnetic field, which can be described by the time constant \( T_2' \).

The over dephasing is thus governed by a time constant \( T_2^* \), which is a combination of \( T_2 \) and \( T_2' \), such that

\[
\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'},
\]

Different water environments/compartment have their own characteristic relaxation times, which are influenced by nearby macromolecules, exchange of water protons, diffusion \textit{etc}. In general, the slower the motion of the macromolecule and the water that interacts with it is, the longer the T2 time. This can range from seconds in the case of cerebral spinal fluid to milliseconds in bones and ligaments.
1.1.6 Bloch Equations

The combination of Eq. (1.55) and Eq. (1.59) is the phenomenological Bloch equation, named after Felix Bloch, who first proposed the equation and demonstrated this NMR phenomenon experimentally (15):

$$\frac{d\vec{M}}{dt} = \gamma\vec{M} \times \vec{B} - \frac{1}{T_1} (M_o - M_z) \hat{z} - \frac{1}{T_2} \vec{M}_\perp$$  \hspace{1cm} (1.61)

When broken down into its Cartesian components under a constant applied field in the $z$-direction, the Bloch equations have the form of

$$\frac{dM_z}{dt} = \frac{M_o - M_z}{T_1}, \hspace{1cm} (1.62)$$

$$\frac{dM_x}{dt} = \omega M_y - \frac{M_x}{T_2}, \hspace{1cm} (1.63)$$

$$\frac{dM_y}{dt} = -\omega M_x - \frac{M_y}{T_2}.$$

Eq. (1.62) is in the same form as Eq. (1.55) whose solution is Eq. (1.56). The solution of the coupled Eq. (1.63) and (64) are

$$M_x(t) = e^{-\omega T_2} \left( M_x(0) \cos\omega t + M_y(0) \sin\omega t \right), \hspace{1cm} (1.65)$$

$$M_y(t) = e^{-\omega T_2} \left( M_y(0) \cos\omega t - M_x(0) \sin\omega t \right). \hspace{1cm} (1.66)$$

These two equations can be combined into the equation describing behaviour of the transverse magnetization in the laboratory frame:

$$M_{\perp}(t) \equiv M_x(t) + iM_y(t) = M_{\perp}(0)e^{i\omega t}, \hspace{1cm} (1.67)$$
where

\[ \varphi(t) = -\omega t + \varphi(0), \tag{1.68} \]

is the phase relative to the laboratory reference frame, and

\[ M_\perp(t) = M_\perp(0)e^{-t/T_2}, \tag{1.69} \]

is identical to Eq. (1.59). Together, Eqs (1.56), (1.65), and (1.66) form a left-handed spiraling trajectory, as the magnetization decays back to equilibrium, and is illustrated in Figure 1.3.

During the decay, the time-varying magnetization field of the precessing proton spins induces a small current in the receiver coil, that is, the changing magnetic flux produces an *emf* in the receiver coil according to Faraday’s law of induction. As seen in Figure 1.3, the recovery of magnetization from the transverse plane follows a spiral trajectory rotating at \( \omega_o \), which means that the flux passing through the receiver coil can be described as a sinusoid with frequency \( \omega_o \), and amplitude enveloped by the exponential decay function with \( T_{2*} \) time constant.
Figure 1.3. Spiral trajectory of magnetization recovery. After the magnetization had been tipped into the transverse plane, it recovers to equilibrium following a left-hand spiral trajectory in the laboratory frame. The x and y-axis are shown offset. As the magnetization, shown in red, follows the blue path, an *emf* is generated in the coil, denoted by the $\Omega$. The *emf* is shown in the upper left as a black trace. This is the basis of MR signal detection.

### 1.1.7 Basic Pulse Sequences

A set of RF and gradient pulses designed to produce a specific form of signal in a receiver coil is called a pulse sequence. Timing diagrams of pulse sequences are typically shown as multiple axis plots versus time. One of the simplest experiments to perform in NMR is the application of a 90° RF pulse to generate a free induction decay (FID) signal. A 90° FID pulse sequence starts by
rotating the net magnetization into the transverse plane with a 90° pulse. The net magnetization
vector is then allowed to precess freely. The resultant FID signal in the rotating frame is

$$s(t) \propto e^{-t/T_2^*}.$$  \hspace{1cm} (1.70)

If the 90° RF pulses sequence was repeated with repetition time TR, the decay curve that
envelops the train of FID signals would depend on T1:

$$s(TR) \propto \rho \left(1 - e^{-TR/T_1}\right),$$  \hspace{1cm} (1.71)

where $\rho$ is the spin density.

The timing diagram for a basic spin-echo sequence is shown in Figure 1.4. Here, a 90° pulse is
first applied to nutate the net magnetization onto the transverse plane. It is then followed by an
180° pulse. Between the 90° pulse and 180° pulse the spin dephases and a FID signal is
generated. After the application of the 180° pulse at time $\tau$, the spins begin to rephase. At time
$t = 2\tau$, the spins return to $\varphi = 0$ and an echo is generated. This time is called the echo time, TE.
However, the magnitude of the echo peak is now reduced due to irreversible dephasing caused
by the T2 relaxation process. The signal equation for multiple spin-echo is of the form

$$s(TE) \propto \rho \left(1 - e^{-TR/T_1}\right) e^{-TE/T_2}.$$  \hspace{1cm} (1.72)

By manipulating TR and TE, different signal weighting can be achieved. For example, if $TE \ll T2$, $e^{-TE/T_2} \rightarrow 1$ and the signal is dominated by T1. This effect is called a T1-weighting. For a
T2-weighted signal, TR is set to a large value (5 times T1) so that $\left(1 - e^{-TR/T_1}\right) \rightarrow 1$ and T2
dominates. A proton density weighted image can be achieved by setting a short TE and long TR.
1.1.8 Magnetic Resonance Imaging

The first MR image was obtained by Lauterbur in 1973 (9), by applying spatially varying magnetic fields to encode spatial information. Three linearly varying magnetic fields, known as gradients, are generated by the auxiliary magnetic field coils, called gradient coils, in three orthogonal directions. The total applied magnetic field at a given point in space ($\hat{r}$) then, is a combination of the main magnetic field, $B_o$, and the linearly varying gradient with components in the $x$-, $y$-, and $z$-directions:

$$\vec{B} = B_o \hat{z} + \left( \vec{G}(\hat{r}) \cdot \hat{r} \right) \hat{z} = B_o \hat{z} + \left( G_{x} x + G_{y} y + G_{z} z \right) \hat{z}$$  \hspace{1cm} (1.73)

where,
These are used to provide spatial localization for pulse sequences described in the previous section. Recall that the Larmor frequency depends on the magnetic field (Eq. 1.51). The gradients introduce magnetic fields which are linearly dependent on the position, leading to a linear relationship between the position and the Larmor frequency. This allows us to tie the frequency of the MR signal to its spatial location, the details of which will be described in the following sections.

### 1.1.8.1 Slice-selection

In two-dimensional imaging, slice-selective excitation can be used to restrict the imaged volume to a user-defined slice. To perform a slice-selective excitation, an RF pulse is applied in the presence of a gradient. Because only the spins precessing at frequencies within the bandwidth (BW) of the applied RF pulse are excited, the thickness of the slice can be selected by altering the bandwidth of the RF pulse, \( BW \), and/or the strength of the slice-selective gradient. For example, the slice thickness in the \( z \)-direction is determined by

\[
\Delta z = \frac{BW}{2\pi \gamma G_z}
\]  

(1.77)
Similarly, the location of the slice can be selected by shifting the centre frequency of the RF pulse.

### 1.1.8.2 Spatial Encoding

The variation in applied gradient fields alters the Larmor frequency within the sample as a function of position, by

\[
\Delta \omega (\vec{r}, t) = \gamma \mathbf{G}(t) \cdot \vec{r}.
\]  

(1.78)

Relative to the signal at \( \vec{r} = 0 \), this results in a phase difference of

\[
\phi (\vec{r}, t) = \vec{r} \int_0^t \gamma \mathbf{G}(t') dt'.
\]  

(1.79)

Let the variable \( \vec{k} \) denote the spatial frequency and set it equal to the function in the square bracket; and we then can write a Fourier relation between the detectable signal \( s(\vec{k}) \) and the spin density, \( \rho(\vec{r}) \):

\[
s(\vec{k}) = \int_\mathcal{R} \rho(\vec{r'}) e^{-i \omega k(\vec{r'})} d^3 \vec{r'}
\]  

(1.80)

From this, an inverse Fourier transform can be performed to reconstruct an image from the MR signal using

\[
I(\vec{r}) = \int_\mathcal{K} s(\vec{k'}) e^{i \omega k(\vec{r})} d^3 \vec{k'}.
\]  

(1.81)

Therefore, it is useful to talk in terms of image space, and \( k \)-space. The Fourier relationship between the two spaces enables the spin density to be reconstructed from the signal when it is sampled over a sufficiently large area of \( k \)-space.
Two strategies can be used for Cartesian spatial encoding with gradients: phase encoding and frequency encoding. In phase encoding, gradient pulses with different strength and/or duration are applied before each signal acquisition so that each acquisition, or 'line', in $k$-space, receives a unique phase. In frequency encoding, the sampling is done in the presence of the gradient, and a 'line' is collected with each point at a unique frequency. In 3D acquisition, phase encoding is applied in two directions. Non-Cartesian acquisition strategies also exist and are beneficial in specific applications.

1.1.8.3 Resolution, Field-of-view, and Acquisition Matrix

Recall from the previous section that the $k$-space is effectively Fourier space and that it represents the spatial frequency information of the object being imaged. This fact means that image details (i.e., high spatial frequency contents) lie in the periphery of $k$-space, while gross features (i.e., low spatial frequency contents) lie in the central region of $k$-space. Therefore, image resolution is determined by how far sampling is performed into the peripheral zone. The larger the $k$-space coverage is, the higher the resolution.

In practice, the resolution (or extent in $k$-space) is determined by two parameters, the field-of-view (FOV), which is the region in image space covered by spatial encoding, and the acquisition matrix size, which is the number of points sampled in $k$-space. Increasing the matrix size while retaining the same resolution (same $k$-space coverage) means that $k$-space is sampled more densely. A fixed resolution means that each pixel in image space still covers the same area, so if the size of the acquisition matrix is increased, there are more pixels in the image space as well,
so FOV must increase. Conversely, the larger the field-of-view in the image domain, the smaller
the spacing between adjacent data points in \( k \)-space. Therefore, FOV is determined by the
spacing of the \( k \)-space samples.

1.1.8.4 Spin-echo Imaging

Spin-echo imaging is an extension of the spin-echo sequence concept previously discussed
(§1.1.7). The timing diagram of a spin-echo imaging sequence is presented in Figure 1.5. First, a
slice-selective 90° RF pulse is applied in conjunction with a slice-selection gradient (tip angle of
other than 90° can also be used, but for the purpose of this discussion, flip angle is limited to 90°
for simplicity). At the half-echo time, a slice-selective 180° pulse is applied in conjunction with
the slice-selection gradient. The phase encoding gradient is usually applied between the 90° and
180° pulses in order to minimize TE, but can also be applied after the 180°. Frequency encoding
is performed after the 180° pulse during the time that the echo is collected. The recorded signal is
the echo. A 'prewinder' is applied between the 90° and 180° pulses. This prewinder gradient is
along the same direction as the frequency encoding gradient to dephase the spins. After the 180
pulse, the frequency encode gradient then rephases the spins at the center of the echo at time TE.
The gradient 'prewinds' the signal to the edge of \( k \)-space by the start of the acquisition of the
echo. The sequence is repeated every TR until sufficient \( k \)-space coverage is achieved. The 180°
RF pulse means that dephasing due to time independent T2′ is rephased after the pulse, making
the sequence less susceptible to T2* effects.
If we were to use multiple $180^\circ$ refocusing pulses (two are shown in Figure 1.5) with sufficiently long repetitions times, we can produce a series of images (assuming single T2 component) with signal intensity that follows

$$s(TE_n) \propto \rho e^{-\frac{TE_n}{T_2}}. \tag{1.82}$$

![Figure 1.5. Timing diagram for basic spin-echo imaging sequence.](image)

The sequence is similar to Figure 1.4 (FID not shown) but with the addition of imaging gradient. A second spin echo is also shown.

In the case where the $180^\circ$ refocusing pulses are imperfect, some of the magnetization remains along the longitudinal axis, which can later be flipped back into the transverse plane by an RF pulse and produces additional unwanted echoes called stimulated echoes. One of the methods to correct for stimulated echo is through the use of Extended Phase Graph, which fits the signal using decay curves simulated with a range of refocusing pulse flip angles (16–18).
1.1.8.5 Gradient-echo Imaging

For maximum signal, spin-echo requires the transverse magnetization to recover to its equilibrium value before the sequence is repeated. For a sample with long T1, the requisite TR lengthens the imaging time considerably. This can be combated by using a smaller flip angle; however, this is at the expense of signal reduction. Instead of using an 180° RF pulse for refocusing as in a spin-echo sequence, gradient-echo pulse sequence uses a gradient pulse for spin refocusing. The lack of refocusing pulse enables shorter TE. However, the use of gradient echo for refocusing makes it much more sensitive to field inhomogeneity. With a spin echo sequence, the effect of T2’ is essentially reversed by the use of 180° refocusing RF pulses. Before the application of the 180° RF pulse, the static field inhomogeneity causes extra spin dephasing. However, after the application of the 180° RF pulse, the magnetization is flipped 180° relative to the static field inhomogeneity, so now it causes extra spin rephasing instead. This cancels out the spin dephasing from before and eliminates the signal’s dependence on T2’. The use of a gradient pulse for refocusing in gradient echo sequences does not reverse this effect.

1.2 The CPMG Sequence

The CPMG sequence is a spin-echo sequence based on the Carr-Purcell (CP) pulse sequence created in 1954 (19). The CP sequence is composed of a 90° excitation pulse followed by one or more 180° refocusing pulses, all applied along the same axis. Because all the pulses are applied along the same axis, imperfection in the refocusing pulses compounds in error with each repetition. Therefore, the sequence is not very robust because perfect flip angles are difficult to achieve due to field inhomogeneity, RF attenuation, hardware limitations, field perturbation from the samples etc.
Meiboom and Gill improved the CP sequence by applying the excitation and refocusing pulses along different axis (20). This has the effect that if the refocusing pulses are identically imperfect, then the even refocusing pulses will undo the imperfection introduced in the odd refocusing pulses (Figure 1.6). This pulse sequence structure of $90^\circ_{x'}-(\tau-180^\circ_{y'}-2\tau)_{N}$ is known as a CPMG sequence.

![Figure 1.6. Meiboom and Gill’s improvement to the Carr-Purcell sequence. By applying the refocusing pulse on a different axis, the CPMG pulse is less sensitive to pulse imperfection. The dephasing disc is shown in red. a) The application of the first imperfect refocusing pulse is shown with the thick blue arrow. b) After the flip, the disc rephases, produces a spin echo, and then dephases, denoted by the blue arrows. c) The application of the next imperfect refocusing pulse returns the disc to the $x'y'$ plane.](image)

1.2.1 Improvements on the CPMG Sequence

An improved version of the CPMG sequence is used throughout the work presented in this thesis, and is based on the work of Poon et al (21). The timing diagram of this CPMG sequence is shown in Figure 1.7. I will be discussing each key features of the improved sequence in turn. The sequence utilizes a slice-selective excitation pulse, after which the signal is phase encoded. Refocusing is then performed using composite pulses bracketed by alternating and descending crusher gradients.
1.2.1.1 Composite Refocusing Pulse

While the application of the refocusing pulse on a different axis avoids the issue of accumulating error with imperfect pulses, this still means that the odd echoes have reduced amplitude. Furthermore, the basic CPMG modification does not account for the fact that imperfect refocusing pulses flip some of the longitudinal magnetization into the transverse plane. To improve the situation, composite pulses were utilized. Specifically, instead of using a straight 180°ₚ refocusing pulse, it was replaced with three pulses: 90°ₓ', 180°ₚ, and 90°ₓ (22).

Imagine the application of an imperfect 90°ₓ that rotates some magnetization in the x'y' plane to just above of the x'z' plane. The follow-up 180°ₚ then rotates this magnetization to the same

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**Figure 1.7. Timing diagram of the improved CPMG sequence.** The sequence uses descending alternating crushers and non-slice selective composite refocusing pulses.
height above the opposite quadrant of the x'z' plane. The imperfect 90°x is applied again and the magnetization ends perfectly at 180° from where it started, identical to that of an 180°y rotation. This shows that the two 90°x pulses are self-compensating.

Now imagine instead that the 90°x are perfect, but the 180°y are not. The first 90°x rotates some magnetization into the x'z' plane. The imperfect 180°y rotates this 180±Δ° around y'. Then, the second 90°x rotates the magnetization back into the x'z' plane. Compared to an 180°y the composite pulse has returned not just a portion but all of the magnetization back into the x'z' plane with a small dephasing error of ±Δ°. This dephasing error is recovered every even echo. If both the 90°x and 180°y are imperfect, we are still left with most of the same the dephasing error in x'y' plane and only a minor loss of magnetization to z'. In any case, these errors are of the second order and much smaller than if a single 180°y were used.

### 1.2.1.2 Crushers

At this point the sequence is quite robust already, but there are still coherence artifacts to deal with. One of these is the production of stimulated echoes. When the refocusing pulse is imperfect, a portion of the magnetization is left in z'. Through subsequent refocusing pulses, some of this magnetization is returned to the transverse plane. That signal is superimposed on the primary echoes and corrupts the T2 weighting. This can be eliminated by using crushers. For the improved CPMG sequence, alternating crushers with descending amplitude were utilized (21).

By bracketing the refocusing pulses with balanced crusher gradients that alternate in polarity between each pulse, the stimulated echo contribution can be eliminated. For magnetization that
remains in the transverse plane after the refocusing, the effect of the crusher gradient before and after the refocusing pulse cancels (matching gradient area). The stimulated echoes, however, only see the crusher while they in the transverse plane. Because every stimulated echo pathway requires the magnetization to be stored in $z'$, they only see one pulse of the crusher gradient pair (either before being stored or after being recalled). Therefore, crushers are effective at eliminating signal contribution from stimulated echoes. Note that in order for the crusher to work for all possible pathways, they need to vary in amplitude so that there is no possibility that the cumulative effect of crushers is nulled over any possible stimulated echo pathway (16). To satisfy this requirement, descending crusher that accommodates the reduction in signal of the primary echoes were used.

Finally, to further improve the sequence, crushers of alternating polarity were used. Consider the magnetization that is dephased and stored in $z'$ after the first refocusing pulse. When part of it returns to the transverse plane after the second refocusing pulse, it experiences the second pulse of the crusher gradient pair. Normally this would undo some of the dephasing, but because the polarity of the crusher gradient is now reversed, the effect of the crusher is now additive.

1.2.1.3 Phase Cycling

Besides stimulated echoes, another coherence artifact is the FID signal artifact. Due to $B_1$ inhomogeneity and/or non-uniform excitation profile, FID signal can arise during the refocusing train. Because each echo is not individually phase encoded in this sequence, whenever the FID signal has significant amplitude during readout, it will contribute to the zero phase signal and show up as a central zipper artifact in the image. Like stimulated echoes, it can be suppressed by
the use of crushers. However, there are situations where the descending crusher may not be sufficiently large to suppress the FID signal, especially at later echoes. To remedy this, phase cycling can be used. By switching the phase of the excitation pulse between two acquisitions, the desired signals are now of opposite sign. However, because the FID signal arises during the echo train, it remains unaffected by phase cycling, and can be removed by subtraction. Phase cycling can also be used to remove baseline/DC offset artifact; however, with modern digital hardware, this is typically a no longer a problem. An example of how phase cycling removes the spurious artifact is shown in Figure 1.8.

![Figure 1.8. Removal of spurious FID artifact by phase cycling.](image)

Left image shows the last echo from a 32 echo acquisition at one average corrupted by a single pixel wide zipper artifact arising from spurious FID signal. The right image is acquired at two averages with phase cycling. Note the increase in SNR and the removal of the zipper artifact. Each pixel is 100 µm × 100 µm.

1.3 The Central Nervous System

The brain and the spinal cord make up the central nervous system (CNS). All bilateral symmetric animals rely on the CNS to integrate and act on the information received from receptors and
sense organs. The brain is protected by the skull, whereas the spinal cord is protected by the vertebrae. Within these bony structures, the entire CNS is enveloped by the meninges composed of three layers: the dura mater, the arachnoid mater, and the pia mater. The dura mater is the outermost meninx and consists of tough white fibrous connective tissue. In the middle is the arachnoid mater, named for the web-like appearance of its delicate connective tissue. The pia mater is the innermost and the thinnest meninx. It adheres to the surface of the spinal cord and brain.

Occupyin the subarachnoid space (between the arachnoid mater and the pia mater) is the cerebral spinal fluid (CSF), a clear bodily fluid that supports the CNS. The CSF suspends most of the mass of the CNS and protects it from mechanical shock. It circulates nutrients and removes metabolic waste (23), provides chemical stability, helps regulate cerebral blood flow, and acts in place of a lymphatic system (which the CNS lacks) for immunological protection (24). It is, however, a nuisance in MR imaging of the CNS, primarily because of image artifacts resulting from CSF pulsation (caused by several factors including vascular pulsation, respiration, and slow frequency waves). When CSF signal is not suppressed, the sharp transitions in image intensity between it and the spinal cord can also lead to truncation artifacts.

1.3.1 The Spinal Cord

A major function of the spinal cord is to relay (ascending) sensory impulses from, and (descending) motor impulses to the peripheral nervous system. It also contains neural circuits for integrating reflexes and central pattern generators for rhythmic movement related to locomotion.
The human spinal cord extends from the medulla oblongata of the brainstem down to the second lumbar vertebra (farther in rat). A pair of spinal nerves arises from each of the spinal cord’s 31 (34 in rat) segments (C1-8, T1-T12, L1-5, and S1-5 versus C1-8, T1-13, L1-6, S1-4, and Co1-3 in rat) (25). The dorsal root consists of sensory fibers and conveys information to the dorsal gray horn; the ventral root consists of motor fibers and receives information from the ventral gray horn.

1.3.2 White Matter and Gray Matter

Looking at the cross section of the spinal cord, one can immediately recognize two distinct nervous tissue organizations, easily distinguishable due to their colouring. The inner, darker coloured portion is made of gray matter (GM), composed of neuronal cell bodies, dendrites, unmyelinated axons as well as some myelinated axons, glial cells, and capillaries. The outer, lighter coloured portion is made of white matter (WM), composed of myelinated axons of sensory/afferent and motor/efferent, as well as propriospinal neurons supported by neuroglia. The fatty content of myelin gives it a whitish colour, hence the name.

Of primary interest to us are bundles of myelinated fibers, or tracts, which run long distances up and down the spinal cord in the white matter, with the descending tracts carrying motor information and the ascending tracts carrying sensory information. The dorsal column is of special interest, because it is conveniently accessible after laminectomy and the opening of the meninges, and, because it contains fairly homogeneous ascending and descending tracts that simplifies the study of Wallerian/anterograde degeneration after a spinal cord injury (see §1.3.4).
1.3.3 Myelin and Myelin Debris

As previously mentioned, axons in WM are covered by fatty myelin sheaths. These long, cylindrically shaped dielectric insulators are required for the proper functioning of the nervous system (26). The relationship between the external diameter of the myelin sheath and the internodal length varies, but typically the internodal length is one to two orders of magnitudes larger than the external diameter of the myelin sheath (27,28). Myelin sheaths are formed of tightly wrapped lipid bilayers with many embedded proteins (such as myelin basic protein, proteolipid protein, and myelin oligodendrocyte glycoprotein). Myelin sheaths are formed by oligodendrocyte in the CNS. Each oligodendrocyte can myelinate up to 50 axons (29). When the cytoplasmic process of the oligodendrocyte wraps around an axon, the cytoplasmic space is eliminated in the formation of compact myelin and the cytoplasmic-facing halves of the membranes comes into contact, forming the major dense line of the myelin sheath. The extracellular space facing halves form the minor dense line. The major dense line, formed of closely spaced cell membranes, appears dark under microscopy. The minor dense line, due to the wider spacing of the cell membranes and the extracellular water trapped in between, appears much lighter.
The composition of myelin, when dehydrated, is approximately 70% lipid and 30% protein (30). Figure 1.9 shows a schematic representation of a typical neuron. Individual sheaths are roughly 1 mm long and spaced 1–2 microns apart at the node of Ranvier (31), and the periodicity of myelin membrane bilayers is in the order of 11 to 12 nm in the CNS (32–34).

Each myelin sheath can be thought of as a capacitor and resistor in parallel. With each additional bilayer, the capacitance of the myelin sheath decreases (analogous to capacitors in series) and its resistance increases (analogous to resistors in series), thereby decreasing current leak and capacitive load to allow for faster conducting velocity and minimum energy loss (31). So rather than having to regenerate the action potential immediately, it now only has to be regenerated at the nodes of Ranvier (saltatory conduction). In fact, the myelinated portions in between the nodes have hardly any ion channels present (35). In order for an unmyelinated axon to perform as efficiently as a myelinated axon, its diameter would have to be 100 to 1,000 times larger.
1.3.4 Spinal Cord Injury

Spinal cord injury (SCI) caused by disease or trauma that alters its normal function often causes permanent changes in strength, sensation, and other functions below the injury site. Injury can be grossly classified as complete or incomplete. In a complete injury, all function below the injury site is lost while in an incomplete injury, some motor and/or sensory function is retained (36). Injury at the cervical level usually results in quadriplegia and an injury at the thoracic level usually results in paraplegia. SCI causes are mostly traumatic in nature, with motor vehicle accidents being the leading cause. There are 86,000 people living with SCI and 4,300 new cases occur each year in Canada alone (37,38). The financial impacts of these new cases are estimated at 2.7 billion dollars a year (39).

SCI, whether inflicted through transection, contusion, dislocation, or other means, results in interruptions to axonal continuity. The proximal segment, the section of axon still connected to its cell body, undergoes a process called retrograde axonal degeneration (dieback), in which the cut end breaks down, but only for a short distance (Figure 1.10). The distal segment, the section of the axon cut off from its cell body, undergoes Wallerian/anterograde degeneration. Unable to maintain itself, the distal segment starts breaking down, first by becoming beaded and then breaks into smaller roughly aligned fragments, which are phagocytized, leaving behind hollow myelin sheaths. Secondarily, either due to the lack of survival signal and/or pro-degenerative signal from the distal segment to the oligodendrocyte, the myelin sheaths break down as well (though nowhere nearly as abruptly) and are eventually phagocytized too (40). The differences between intact myelin and myelin debris are shown in Figure 1.11.
Throughout this thesis, the term myelin debris will be used to describe non-normal-appearing myelin, including empty, and collapsed myelin sheath, as well as any fragments of the myelin sheath that still appear to retain their unique compact lipid bilayer structure.

Figure 1.10. Axonal degeneration. Figure shows the gradual degeneration of the axon as it breaks down and collapse into smaller portions that are cleared over time. The destruction of the cytoskeleton leads to the beading pattern shown (41).
Figure 1.11. Degeneration of myelin into myelin debris. Top row illustrates intact myelin; bottom row illustrates myelin debris 3 weeks after injury. The first two columns are cartoon illustrations. The third column shows low resolution electron micrographs. Note that the vertical scale is extremely compressed in the first column. As the axon degenerates, the myelin sheaths are no longer maintained and they collapse into irregular shapes and lose their strong orientation. The debris is then gradually cleared.

1.3.4.1 Dorsal Column Transection Injury in Rat Model

There are many rat injury models available, among the basics are transection, contusion, and dislocation, and distraction. Clinically, nearly half of SCI are caused by vertebral dislocation (42); however, transection is the most prevalent in rat studies (43). Transection models have the advantage that they are easy to perform, highly reproducible, and spare non-targeted white matter. For the studies presented in this thesis, a dorsal column (DC) transection (Tx) injury model at the cervical level were used (44,45). The relevant anatomy is shown in Figure 1.12.
**Figure 1.12. Illustration of the axial cross section of rat cervical spinal cord.** On the left, the dorsal column is highlighted by the line pattern. The corticospinal tract, highlighted in blue, is a descending tract. The *fasciculus gracilis* is highlighted in green. It is an ascending sensory tract and carries mainly afferent axons from the hindlimbs. The *fasciculus cuneatus*, highlighted in red, carries afferent axons from the forelimbs. A myelin water fraction map taken at 5mm cranial to C5 8 at 8 weeks post dorsal column transection injury is shown on the right.

The procedure used throughout this thesis is described in Chan *et al* (45). To perform the DC Tx injury, the rats were deeply anaesthetized, placed in a stereotaxic surgical frame in the prone position with their skin on the neck shaved and disinfected. A longitudinal midline incision of the neck was followed by a midline split of the neck muscles to expose the cervical vertebrae. A laminectomy of the C5 lamina was performed with a small rongeur. A Kopf stereotaxic wired knife (model 120; David Kopf Instruments, Tujunga, CA) was secured onto the stereotaxic frame and moved 0.9 mm lateral to the midline on the animal’s right side. The wired knife was lowered 1.1 mm into the dorsal horn between the dorsal roots of C4 and C5 after prepuncturing the dura mater with a needle. The wire knife blade (1.8 mm curvature length) was then extended into the spinal cord through the prepuncture to a further depth of 0.5 mm (total 1.6 mm). The wire knife was drawn up while gently pushing the dorsal column down against the blade with a cotton swab. To ensure that all axons in the dorsal column are cut, a no. 11 scalpel blade was used to sever all axons against the wire knife.
After the DC is cut, axon dieback and Wallerian degeneration occur. Myelin sheaths break down following axon degeneration and the myelin debris is gradually cleared; however, the process is slow, taking up to several months (46). Of special interest to us is the *fasciculus gracilis* (location shown in Figure 1.12), because the majority of this thesis focuses on MWF measurement in this region. Based on our previous findings using the DC Tx model (47), we expect exclusively myelin debris at 3 weeks post-injury which is partially cleared by 8 weeks post-injury in the *fasciculus gracilis* at 5 mm cranial/distal to the injury site (illustrated below in Figure 1.13).

**Figure 1.13.** Cartoon illustration of myelin degeneration in the *fasciculus gracilis*. Normal depicts normal appearing myelin sheaths; 3 weeks depicts complete degeneration into myelin debris at 3 weeks post-injury; and 8 weeks depicts the clearing of this debris by 8 weeks post-injury. There is a reduction in lipid bilayer content through all three time points, although it is most evident at 8 weeks post-injury.

### 1.4 Multi-component T2 Relaxation for Assessing Myelin Content

In MR imaging, an image voxel usually contains multiple T2 components due to the inclusion of multiple water compartments/environments. This is especially true in complex tissues, such as
the spinal cord. In 1978, Vasilescu et al. published their discovery of three distinct T2 components in frog sciatic nerve in NMR experiments using the Carr-Purcell-Meiboom-Gill (CPMG) sequence (48). This was the first time multi-component T2 had been observed in myelinated nerve. It was not until 1991 that the fast relaxing component (T2 = 12.7 ms) was attributed to water trapped between the myelin lipid bilayers (myelin water) (49).

In general, there are three detectable components in MR signal of the CNS: a long T2 component attributed to CSF, an intermediate component attributed to intra/extracellular water, and a short T2 component attributed to myelin water (49–51). The most important parameter to arise from the analysis of multi-component T2 data in WM is the myelin water fraction (MWF), which is the fraction of the signal from myelin water over the total water signal, and has been shown to correlate well to myelin content measurements from other methods (31,52–57). Thus, MR provides an invaluable tool in assessing myelin content in vivo.

The modified CPMG sequence introduced in §1.2 is designed to produce a series of T2-weighted images for quantitative T2 analysis (21). Ideally, the sequences produce purely T2-weighted signal decay curve at each voxel, which can be considered as the sum of single exponential decays from a number of unique T2 components. That is

\[ s(TE_n) \propto \sum_i \rho_i e^{-TE_n/T_{2i}}. \] (1.83)

The standard way to tease out the magnitude of each component is to fit the signal to a large number of (typically logarithmically spaced) exponential decays at many T2 times using non-
negative least square (NNLS) fit that is modified with a smoothing function (regularization) (58).

The algorithm minimizes both the $\chi^2$ and an energy constraint by minimizing

$$\chi^2 + \mu \sum_i s_i^2,$$

(1.84) where $\mu \geq 0$. The size of $\mu$ determines the amount of smoothing. The use of regularization is necessary for producing consistent fits in the presence of noise (59). An example of NNLS fitting is shown in Figure 1.14. MWF is calculated from the resulting T2 distribution by dividing the integral of the myelin water peak by the total integral of the T2 distribution.

$$y(t) = \sum_{i=1}^{M} s_i e^{-t/T2_i},$$

Figure 1.14. Example of multi-exponential T2 fitting with NNLS. Data acquired from the dorsal column of a paraformaldehyde fixed rat spinal cord sample using a 4 turn solenoid coil is shown (TE/TR = 6.738/1500 ms, 1 mm slice thickness, 100 µm in-plane resolution, 6 average). The top graph shows the data points and the fitted curve. The bottom graph shows the regularized T2 distribution exhibiting the characteristic myelin water peak and intra/extracellular water peak.
Note that the T2 times shown in Figure 1.14 are shorter than some literature values because the sample was scanned in fixative, which lowers the T2 relatively to samples that had had the fixative removed through successive washes in saline and then scanned in saline. Furthermore, the separation of T2 between the two components were only a factor of two, which typically leads to difficulties in obtaining reliable peak separation, especially in view of a relatively low SNR, because the decay is captured by relatively few echoes. However, in practice, the experimental setup used in this thesis for ex vivo work is capable of produces a first echo SNR of greater than 700 with reasonable scan time, so good peak separation is achievable for the two components shown in Figure 1.14. Additionally, as outline in §1.2, stimulated echo contamination had been carefully suppressed.

Another useful parameter that can be derived from the T2 distribution is the geometric mean T2 (GMT2). For a range of T2 from $T2_{\text{min}}$ to $T2_{\text{max}}$, the GMT2 is defined as

$$ \text{GMT2} = \exp \left[ \frac{\sum_{T2_{\text{min}}}^{T2_{\text{max}}} S(T2) \log(T2)}{\sum_{T2_{\text{min}}}^{T2_{\text{max}}} S(T2)} \right], $$

(1.85)

where $S(T2)$ is the amplitude of the T2 distribution. Because the T2 distribution is calculated on a logged T2 scale, GMT2 provides not only a convenient way to calculate the T2 of each component, but also the mean between multiple components. The approach works especially well when the T2 components are lognormal (that is normally distributed on a logarithmic scale), as is often the case. The use of geometric mean also ensures that the geometric mean of R2, defined as $1/T2$, agrees with $1/\text{GMT2}$, which is not the case for simple arithmetic means.
1.5 Other Methods for Assessing Myelin Content

Beyond the quantitative T2 method introduced in the previous sections, there are other MR based methods that are proposed to reflect myelin content, including magnetization transfer (MT), diffusion, T1 relaxation, and mcDESPOT (multi-component driven equilibrium single pulse observation of T1 and T2). This section gives a brief overview of each method.

1.5.1 Magnetization Transfer

MT imaging utilizes the exchange of magnetization between nonaqueous tissue and water (60). The technique involves applying an off-resonance pulse to excite the nonaqueous tissue. The signal decay on the nonaqueous tissue itself is too fast to be readily detected. However, because the two pools are in contact the water signal is reduced through magnetization transfer. The amount of transfer can be characterized by the magnetization transfer ratio (MTR). MTR is calculating as the ratio of the difference between images acquired with, and without a MT pulse, to the image acquired without a MT pulse. In WM, MTR correlates to myelin content. MT can be made more quantitative by fitting models of magnetization exchange to the data to extract tissue parameters, such as a two-pool model that allows the calculation of the nonaqueous and water pool size, and their respective T1, T2, and exchange rate (61). However, while MT is a very sensitive measure and correlates relatively well to myelin content, it is not very specific because it is influence by other factors. For example, MTR is influenced by inflammation, axonal density, iron content, total water content, and pH, making multi-component T2 analyses much more specific in comparison (57,62,63). Quantitative models, however, can improve on this lack of specificity.
1.5.2 Diffusion

The use of diffusion tensor imaging (DTI) is another technique that has been used extensively to study the CNS. Diffusion tensor imaging (DTI) gives a measure of the restricted diffusion of water in tissue, describing both its rate and direction. It is especially useful in the CNS because the organization of the tissue (myelinated and unmyelinated fibre bundles) lends itself well to DTI. Typical, the transvers diffusivity ($D_{\text{trans}}$, diffusivity transvers to the fibre), longitudinal ($D_{\text{long}}$, diffusivity parallel to the fibre), apparent diffusion coefficient (ADC), and fractional anisotropy (FA, how isotropic the diffusion is) are calculated. FA and ADC correlates to both axon density and myelin count (64). $D_{\text{long}}$ was found to correlate well with the amount of axon (65,66). However, while $D_{\text{trans}}$ had been proposed to reflect myelin content, follow-up studies suggest otherwise (57,67–69), making its utility in myelin quantitation questionable.

1.5.3 T1 Relaxation

Similar to multi-component T2, the same type of analyses can be applied to purely T1 weighted sequences to quantify longitudinal relaxation in WM (70). However, while multiple T2 components in WM are consistently observed, T1 is typically reported as a single component. When two T1 components are measured, however, the short T1 component is attributed to myelin and MWF is calculated in a similar fashion. One possible mechanism that gives rise to the two T1 components magnetization exchange between the nonaqueous tissue and water (71). The technique is promising, but currently not very robust and suffers from low SNR efficiency.
1.5.4 mcDESPOT

mcDESPOT is an extension of driven-equilibrium single-pulse observation of T1 and T2 sequences (DESPOT1 and DESPOT2) for multi-component T1 and T2 quantitation (72). One of the attractions of the technique is that DESPOT1 and DESPOT2 are based on two clinical standard sequences: spoiled gradient echo (SPGR) and balanced steady state free precession (bSSFP). A combination of SPGR images are used to generate a T1 signal curve; while a combination of bSSFP images are used to generate a T1 and T2 dependent curve. One can then derive T2 measurements from bSSFP given the T1 measurements from SPGR. In mcDESPOT, a two-pool model, consisting of myelin water and intra/extracellular water, can be fitted to the data produce the T1, T2, and the residence time of each pool, as well as the MWF and intra/extracellular water fraction. Compare to multi-component T2, mcDESPOT tends to produces significantly MWF measurement (perhaps due to MT effect), is experimentally more complicated, and the required use of complex models to fit the data affects its reproducibility (73).

1.6 Overview of the Thesis

As mentioned before, the main body of this thesis is divided into three parts: the first part deals with improving the speed of MWI using compressed sensing, the second part deals with the effect of fixation on MWI, and the last part deals with MWI in the presence of myelin debris. The investigation into MWI in the presence of myelin debris was motivated by the fact that there are currently no MR methods capable of distinguishing between intact myelin and myelin debris. Light microscopy based studies have suggested that MWF measures both intact and myelin and myelin (52,74,75). However, the relationship between MWF and myelin debris, including the
potential effect of the morphological changes in myelin debris, was unclear. The studies presented in the last part of the thesis were designed to remedy this. The first part of the thesis is spurred on by the fact that MWI using multi-echo sequence produces lengthy scan time. Scan time is of special importance in clinical settings. Shorter scan time not only improves throughput, but also patient comfort, cost, etc. In the next chapter, I will introduce the concept of compressed sensing and show that it can be used to improving the speed of MWI.
Chapter 2: Compressed Sensing CPMG for Myelin Water Imaging

Myelin content is an important marker for central and peripheral nervous systems pathology, because it is affected by many diseases and injuries. Myelin water imaging (MWI) has been shown to measure myelin content in normal and diseased brain and spinal cord tissue (53,74,76). One way to generate a myelin water map is to utilize the multi-echo Carr-Purcell-Meiboom-Gill (CPMG) sequence to obtain T2 decay curves of the tissue and fit them for the individual T2 components (77). The resulting distributions are used to generate myelin water fraction (MWF) map that correlates well to the amount of myelin measured by histology (53,74). CPMG is inherently a slow sequence (73), especially for in vivo small mammal studies with conflicting requirements of high signal-to-noise ratio (SNR) and spatial resolution, and reasonably short scan time limited by adverse effects of prolonged anaesthesia. While as gold standard CPMG yields very good MWF maps, it is too slow for many practical applications.

There have been several studies that proposed pulse sequence based techniques and parallel imaging based methods for fast T2 imaging. For example, Mädler et al. (78) have demonstrated the use of gradient and spin echo based sequence for accelerated MWI acquisition by sampling the periphery of k-space using gradient echoes. This is because most of the T2 weighting of the image resides primarily in the central k-space (low frequency), so the less accurate gradient echoes offer a minimal trade off in the periphery.

More recent works (79,80) combine fast pulse sequences with parallel imaging methods to reduce the data acquisition time for T2 imaging. The multi-echo fast spin-echo sequence used in
(79,80) samples the center of the $k$-space accurately with even echoes and the periphery with less accurate odd echoes to retain CPMG like sampling at the center of the $k$-space. This was combined with a multi-coil parallel imaging technique to accelerate the scan time further.

Compressed sensing (CS) offers another approach to accelerating data acquisition. In (81) a novel method was proposed to reconstruct T1 and/or T2-weighted images. Instead of using transform-based dictionaries such as wavelets, it posits using an empirical learned dictionary composed of atoms (linear combination of basic elements, including the definition of the elements themselves) that emulate the actual physical process of T1/T2 relaxation. Even though (81) showed improvements over standard CS based reconstruction, it has several limitations. The major limitation is in designing the empirical dictionary. To date, all the studies in dictionary learning are heuristic, because such an explicit matrix requires large storage, and unlike fast operators such as wavelets, is difficult to operate with. Even though such explicit dictionaries become richer as more atoms are added (leading to better reconstruction), the storage and computational requirements limit their size. The only option is to manually tune the dictionary so as to balance efficiency with accuracy. The other problem is that ‘learning’ the dictionary and estimating the image simultaneously is a highly non-convex problem. Therefore, unlike standard CS, such methods do not have any theoretical guarantees of optimality (that is, the solution cannot easily be verified as the optimal one).

A different extension to standard CS approach is to exploit redundancy in image space by utilizing spatial and temporal correlations (82). Majumdar et al. have proposed a method for accelerating multiple acquisitions of the same anatomy in MR by utilizing both the intra-image
spatial redundancy and inter-image correlation (83). For MWI using a CS CPMG sequence, time saving can be realized by jointly reconstructing all the echo images simultaneously while enforcing group-sparsity. This formulation makes echo images reconstructed with high accuracy from significantly fewer $k$-space samples possible. Despite the non-linear nature of MWF map generation, the assumption is that if the series of individual T2-weighted echo images can be reconstructed accurately, the resulting parametric map (i.e. the MWF map) will be accurate as well. We hypothesize that using CS multi-echo CPMG with group-sparse reconstruction will increase the acquisition efficiency of myelin water images. Both simulated undersampling and actual undersampling experiments were performed.

### 2.1 Theory

Compressed sensing (CS) is a signal processing technique that exploits signal sparsity in order to acquire and reconstruct a signal beyond the Shannon-Nyquist limit. It can be thought of as the reverse of signal compression. Where normal compression acquires the entire signal and then compress it, compressed sensing samples the signal in an already “compressed” manner. As long as the signal is sparse in some domain and its measurement is incoherent in said domain, recovery of the original signal is possible. An example of sparse MR data in the wavelet domain is shown in Figure 2.1.
2.1.1 Group-sparsity

For a series of T2-weighted echo images, the contrast between two tissues may be high in some weighting, while lower in others. But since the underlying anatomy is the same, the positions of the tissue boundaries (edge) that create the contrasts are also the same. Wavelet transform effectively encodes these edges in an image, with a stronger edge correlating to a higher wavelet coefficient. In homogeneous areas, the wavelet coefficients are zero. What is of importance is that the position of the high valued wavelet coefficients will remain the same for all the T2-weighted echo images. Suppose, we represent the $k$-space data by

$$ y_i = R_i F x_i + \eta_i, \quad i = 1..T. $$

where $y_i$ is the $k$-space sample for the $i^{th}$ T2-weighted image $x_i$ (this is the vectorized version of the 2D image), $F$ is the Fourier mapping from the image space to the $k$-space and $R_i$ is the under-sampling mask used for acquiring the $i^{th}$ T2-weighted echo image. The problem is to reconstruct
the T2-weighted images \((x_i)'s\) given their \(k\)-space samples \((y_i)'s\). CS-based reconstruction assumes that the image is sparse in the wavelet domain. Following Majumdar et al. (5), we are using orthogonal wavelets in this work, so that Eq. 2.1 can be represented in terms of wavelet coefficients as,

\[
y_i = R_{FW}^T \alpha_i + \eta_i, \quad i = 1...T,
\]

where \(W\) is the wavelet transform (\(W^T\) is its inverse since orthogonal wavelets are being considered) and \(\alpha_i\) is the wavelet coefficient for the \(i^{th}\) image. The \(k\)-space data acquisition model in Eq. 2.2 can be represented as follows,

\[
y = \Phi \alpha + \eta,
\]

where \(\alpha = \begin{bmatrix} \alpha_1 \\ \vdots \\ \alpha_T \end{bmatrix}, \eta = \begin{bmatrix} \eta_1 \\ \vdots \\ \eta_T \end{bmatrix}\). Let the images be of size \(N \times N\). Because the wavelet transform considered here is orthogonal, the wavelet coefficient vector for each image \(\alpha_i\) is \(N^2\). The coefficients of the vector \(\alpha\) in Eq. (2.3) can be grouped according to their positions. If we consider the \(T\) coefficient vectors \(\alpha_{1,r}, \ldots, \alpha_{T,r}\) as one group, then all the coefficients in the vector \(\alpha\) form \(N^2\) groups, each group having \(T\) coefficients.

It has been argued by Majumdar et al. (83) that, when the wavelet transform coefficients are grouped by their indices, each group will either have high valued coefficients or very small (nearly zero) valued coefficients. As the wavelet coefficients of the individual images are sparse, the combined vector \(\alpha\) is group-sparse when grouped by the indices. Majumdar et al. (5)
proposed incorporating the group-sparsity into the reconstruction problem via the following mixed $l_{2,1}$-minimization,

$$\arg\min_{\alpha} \|\alpha\|_{2,1} \text{ subject to } \|y - \Phi \alpha\|_2 \leq \sigma,$$

(2.4)

where $\|\alpha\|_{2,1} = \left( \sum_{k=1}^{K} \left( \sum_{r=1}^{R} \alpha_{k,r}^2 \right)^{1/2} \right)^{1/2}$.

The $l_2$-norm $\left( \sum_{k=1}^{T} \alpha_{k,r}^2 \right)^{1/2}$ over the groups of correlated coefficients $a_{k,r}$, $k = 1 \ldots T$ promotes a dense solution within the group $(k)$, whereas the summation over the $l_2$-norm enforces group-sparsity, i.e. the selection of only a few groups.

2.2 Methods

Three fully sampled CPMG datasets were used as baselines for simulated undersampling: a) a computer generated electronic phantom designed to mimic an idealized excised rat cervical spinal cord, b) a rat cervical spinal cord ex vivo, and c) rat lumbar spinal cord in vivo (Figure 2.3). Data for b) and c) were from a previous study (7,84). An excised rat cervical spinal cord from the same study was used for actual CS CPMG acquisitions.

2.2.1 Electronic Phantom

The electronic phantom, shown in Figure 2.3 (middle), was modelled after an excised rat cervical spinal cord sample constructed of idealized gray matter (GM), white matter (WM) and fixative solution. Only T2 relaxation was considered. The T2 values used reflect those typically seen in our ex vivo setup at 7T outline in the next section. The fixative was made of a single pool with
T2 of 1,500 ms; the gray matter was made of a single pool with T2 of 24 ms; and the WM was made of two pools; 35% was assigned T2 of 12 ms and 65% with T2 of 28 ms. Proton densities were set at 34.4, 32.9, and 30.2 respectively. Data was generated at 25 µm isotropic resolution and resampled to 100 µm × 100 µm by 1 mm slice to match the *ex vivo* baseline. The advantage of using synthetic data is that it is uncorrupted by noise, which allows us to evaluate the effect of CS reconstruction artifacts on its own. However, the electronic phantom’s geometry and T2 distribution are simplistic.

### 2.2.2 Ex Vivo Imaging

Previously acquired data from paraformaldehyde-fixed C4/5 spinal cord sample excised from female Sprague-Dawley rat was chosen for this study (45). The cord was scanned on a 7 T/30 cm bore preclinical MRI scanner (Bruker, Germany) using a 13 mm inner-diameter and 25 mm long transmit/receive solenoid coil. A single slice multi-echo CPMG sequence (21) was prescribed at 5 mm cranial to DC Tx injury with 256 × 256 matrix size, TE/TR = 6.738/1500 ms, 32 echoes spaced 6.738 ms apart, 2.56 cm field-of-view (FOV), 1 mm slice, number of averages (NA) = 2, 4, and 8, and the excitation pulse phase cycled between 0° and 180°. Acquisition time was 50 minutes. Another spinal cord sample obtained under the same protocol was used for the actual CS CPMG acquisition. The chosen TE is the shortest achievable by the setup at this resolution. It is mainly limited by the RF power delivered to the coil over the short (1 ms) excitation pulse. Only 32 echoes were acquired because at a TE of 6.738 ms, any additional echoes would be sampling the noise floor and offer no improvement in MWF map quality.
2.2.3 **In Vivo Imaging**

Data from lumbar spine acquired using surgically implanted coil (85) at T12/L1 level was chosen from a previous study (84). The use of implantable coil enables an SNR gain relative to single element surface coil (86,87). The same CPMG sequence used to acquire the *ex vivo* data was used here with a slice thickness of 1.5 mm, in-plane resolution of 117 μm, and NA=6. The acquisition was respiratory triggered to minimize motion artifacts, which resulted in a total acquisition time of approximately 45 minutes.

2.2.4 **Simulated CS Data**

*k*-space data, undersampled in the phase encode (PE) direction, was generated from each data set for different acceleration factors (1.33, 1.5, 1.6, 2, and 4, corresponding to 192, 170, 160, 128, and 64 PE lines). One-third (33%) of the read-out lines were placed around the centre of the *k*-space, and the rest distributed uniformly at random in the periphery. Different sampling patterns were used for each echo to utilize the benefit of group-sparse optimization (83). Reconstruction was performed via group-sparse synthesis prior method using Spectral Projected Gradient for L1 (SPGL1) package (88). Daubechies 8 wavelet at three levels of decomposition (while the maximum levels of decomposition is \( \text{log}_2(\text{size})-1 \), three levels works the best in practice) was used as the sparsifying transform. Wavelet transform and total variation are combined to reconstruct the image (82,89). Simple zero-filled (ZF) reconstructions of equivalent PE lines were also performed for each case.
2.2.5 Compressed Sensing CPMG Sequence / Actual CS

The single slice CPMG sequence was modified to acquire undersampled data using the same sampling scheme as in simulated undersampling. Gradient values were sorted such that PE jumps are minimized to reduce eddy current artifacts. Echo spacing was unaffected. The sequence diagram is shown in Figure 2.2. CS CPMG data was acquired from uninjured *ex vivo* cord at various acceleration factors and numbers of averages (NA = 8 with acceleration factor of 1, and 2; NA = 4 with acceleration factor of 1, 1.33, 1.5, and 2; and NA = 2 with acceleration factor of 1, 1.33, and 1.5).

![Sequence Diagram](image)

**Figure 2.2. Timing diagram of the 2D CS CPMG sequence.** The sequence uses descending alternating crushers and non-slice selective composite refocusing pulses. Each echo is phased encoded individually using a pair of gradients of opposite polarity around the echo signal. The phase encode gradients are sorted to minimized gradient amplitude jumps between each echo.
2.2.6 Data Processing and Analyses

The data was processed with software procedures developed in-house using MATLAB R2009b (MathWorks Inc., Natick, MA). Regularized non-negative least square analysis (77) was used to calculate the continuous T2 distributions that have minimal energy within $\chi^2$ misfit of 2% to 2.5%. MWF maps were generated by dividing the integral from 6.738–20 ms by the total integral of the T2 distribution for each voxel. Normalized mean square error (NMSE), defined here as

$$NMSE = \frac{\|x - x_{\text{ref}}\|}{\|x_{\text{ref}}\|},$$

(2.5)

where $x$ is the CS images and $x_{\text{ref}}$ the fully sampled images, was used as the metric for comparing the results. NMSE were generated from the spinal cord voxels for a) all the T2-weighted echo images, b) first T2-weighted echo image, and c) the MWF map. NMSE was chosen because it is sensitively to both systematic bias and random error; it tends to highlight infrequently appearing large differences; and it amplifies small difference because the absolute differences are summed.

The use of volume solenoid combined with the small sample size did not require the use of stimulated echo correction.

2.3 Results and Discussion

Figure 2.3 shows the NMSE values at various simulated undersampling as a function of the number of PE lines used (256 being fully sampled) for the electronic phantom, *ex vivo* data, and *in vivo* data. The same trend in NMSE was observed in all three tested SNR levels (SNR = 786 at NA = 8, SNR = 585 at NA = 4, and SNR = 397 at NA = 2) in the *ex vivo* cord. It is important to note that while the NMSE of the T2-weighted echo images were drastically lower in the
electronic phantom than the ex vivo and in vivo cases, its MWF NMSE were comparable. This suggests that the reduction in MWF map quality with increased acceleration is largely a result of CS reconstruction and is less affected by data quality.

While the quality of CS reconstructed T2-weighted echo images remained excellent with increased acceleration factor, so did the MWF map. The first and eighth T2-weighted echo images and MWF map, as well as the difference image to the baseline for each, is shown in Figure 2.4-5, along with ZF reconstruction of equivalent PE. Qualitatively, the MWF map remained excellent when acceleration factor is under 2×. Interestingly, NMSE in the electronic phantom also decreased sharply when the acceleration factor was reduced below 2× as seen in Figure 2.3, matching the above observation. In general, at and above 2× acceleration, ZF outperforms CS and offers faster image reconstruction; however, neither methods produce usable MWF at these accelerations due to artifacts. The enhancement in the corticospinal tract shown in Figure 2.6 was clearly delineated. Especially in the case of the electronic phantom, the majority of NMSE in MWF were contributed by GM/WM interface, where high wavelet coefficient corresponds to strong edge information. However, errors were smaller than in non-CS images with equivalent PE at reduced FOV. While these regions are typically avoided in ROI analyses of WM tracts due to partial volume effects, the higher spatial resolution allows for a more precise ROI definition. The trend in NMSE did not change when the GM/WM boundaries were excluded from the analyses. NMSE also did not vary significantly between WM tracts. The salt and pepper noise (sparsely occurring bright and dark pixels relative to the background) in the difference maps of the non-synthetic baselines was a large contributor to NMSE in both the CS and ZF reconstructions. In the CS case, it was likely a result of the total variation minimization.
The *in vivo* test case did not have the necessary SNR to benefit from CS undersampling. Figure 2.7 and Figure 2.8 show the result of actual CS CPMG acquisition. Again, MWF map quality remained excellent when acceleration factor was under 2×. The result did not appear to suffer from any eddy current artifacts. There was no significant systemic difference in the MWF maps in any CS accelerated data. The mean error of MWF map between the accelerated and the fully sampled datasets never exceeded 0.01. Note that it is the absolute difference shown in the figures, and the periodic nature of the CS artifact tends to average to zero.

**Figure 2.3. NMSE at various simulated undersamplings.** NMSE was calculated from voxels in the spinal cord against the fully sampled baseline images (256 phase encodes) and plotted against the number of phase encoding lines used. NMSE was generated from the MWF map, all the T2-weighted echo images, and the first T2-weighted echo image. Insets show the first T2-weighted echo image of each baseline. Note that while the NMSE in the T2-weighted echo images were very low in the electronic phantom, its MWF NMSE were comparable to that of *ex vivo* and *in vivo* data.
Figure 2.4. Reconstruction results from simulated undersampling of the electronic phantom. The first and eighth T2-weighted echo image, and the MWF map are shown in rows with their absolute differences against the fully sampled baseline image (far right column) shown immediately below. CS and ZF indicate reconstruction type. The numbers on top indicate the phase encode lines used and the equivalent acceleration factors. Vertical bar on left-hand side shows colour map scaling.
Figure 2.5. Reconstruction results from simulated undersampling of the *ex vivo* baseline. The first and eighth T2-weighted echo image, and the MWF map are shown in rows with their absolute differences against the fully sampled baseline image (far right column) shown immediately below. CS and ZF indicate reconstruction type. The numbers on top indicate the phase encode lines used and the equivalent acceleration factors. Signal region in T2 image is 4 mm in diameter (cropped). Vertical bars show colour map.

Figure 2.6. Reconstruction results from simulated undersampling of the *in vivo* baseline. The first and eighth T2-weighted echo image, and the MWF map are shown in rows with their absolute differences against the fully sampled baseline image (far right column) shown immediately below. CS and ZF indicate reconstruction type. The numbers on top indicate the phase encode lines used and the equivalent acceleration factors.
Figure 2.7. MWF maps generated from actual compressed sensed CPMG data *ex vivo*. Odd number rows show the MWF map and even number rows show the absolute difference image against the baseline MWF map. The numbers on top indicate the phase encode lines used and the equivalent acceleration factors. Number of averages (NA) are indicated on the left-hand side. MWF quality remained adequate at 170 phase encode lines (1.5× acceleration) at two averages, which had a total acquisition time of 8 minutes. Even averages were used to allow the use of phase cycling.
For accurate myelin water measurement, an SNR in the first echo image of greater than 200 is normally required without filtering (90,91). As hardware and methods are refined, SNR per acquisition rises and fewer signal averages are required. The protocol used in this study for ex vivo rat spinal cord imaging is capable of generating an SNR in the first echo image of ~400 with two averages, which implies that one average should be sufficient if the spurious FID signal generated by imperfect refocusing pulses is suppressed by the use of alternating descending crushers. In the 2D, ex vivo cases, we had excellent in-plane field homogeneity and sufficiently large crushers that we could acquire excellent decay curves without the use phase of cycling. However, at a very high spatial resolution (or in other less ideal conditions), the required crusher strength to achieve $2\pi$ phase dispersion across the voxel is difficult to fulfill (92), especially for late echoes. Lastly, the strong gradient pulses can also cause significant eddy currents. One
solution is to use a two-step phase cycle, where the phase of the excitation 90° pulse is alternated between 0 and 180°, and the signal is alternatively subtracted and added. Such phase cycling effectively eliminates the spurious FID signal generated by the imperfect refocusing pulses, but requires at least two averages. Thus, CS CPMG is useful in achieving a shorter scan time than traditionally possible while retaining the use of phase cycling.

The added flexibility in acquisition time by CS CPMG allows for larger coverage, higher resolution, and additional imaging methods in limited scan time. For *ex vivo* scenarios, scan time is typically less important a consideration, so CS CPMG is of little benefit here. However, when histological comparison to high-resolution electron microscopy is desired, scan time may be limited by the fixation procedure (for the whole story, see Chapter 3).

While MWI has been successfully applied in excised rat spinal cord (74,93), the high SNR and spatial resolution requirement make it difficult to apply *in vivo*. In order to achieve the high SNR *in vivo*, the slice thickness was increased to 1.5 mm. The drawback is that this increases partial volume effect in the cranial/caudal direction. Fortunately, the cord was fairly uniform in the imaged section and efforts were made to position the animal such that the spin lies straight. Despite this, increased partial volume effect was observed, evident as poor tissue boundary definition when comparing Figure 2.8 to Figure 2.6. The increased voxel volume, combined with six scan averages, gave us the necessary SNR; however, with respiratory gating, the acquisition took approximately 50 minutes. If we then include the setup time, the animal would be under anaesthesia for over an hour just to cover a single slice. The data shown in Figure 2.8 was acquired over a two-hour experiment. While imaging time is less of an issue with *ex vivo*
experiments, CS can be used to reduce sample heating from RF energy absorption in high-powered sequences.

The CS sampling scheme used here can be further optimized. In this study, each echo is phase encoded using a pair of PE gradients of opposite polarity around the echo signal, although the optimal solution is to use a single PE gradient pulse to encode all the echoes to avoid phase encoding the spurious FID signal (21). Moving to other $k$-space trajectories, such as radial acquisition, can also improve image quality. Furthermore, generating a new randomized gradient table each time does not guarantee the optimal sampling scheme. An exhaustive search should be used to determine the best sampling scheme (82). Visual quality may be improved by moving to more sophisticated wavelet dictionary. When phase cycling is used, instead of signal averaging before CS reconstruction, the dataset can be formulated as a single CS reconstruction problem. Regardless, the Cartesian based sampling scheme and reconstruction method used in this study is easy to implement on any scanner and applies equally well to other MWI methods, such as gradient echo and spin echo (GRASE) based myelin water measurement (78,94). Because patient comfort and cost are always of concern in the clinical setting, reduction of scan time can be beneficial, especial since MWI has been applied successfully in clinical settings, such as in monitoring the demyelination process in multiple sclerosis (53,54).

For a fixed TR, the SNR is proportional to the square root of the acquisition time, so that the SNR efficiency decreases as acquisition time increases. The NMSE shown here also decreases with increasing acquisition time, and time-normalized NMSE follows an even steeper decline.
Therefore, CS multi-echo CPMG with group-sparse reconstruction can have large gain beyond the traditional minimum averages.

While the generated MWF maps consistently produce NMSE that is of an order of magnitude larger than the NMSE of the source T2-weighted echo images, the behaviour is typical of MWF map generation. The NMSE in the T2-weighted echo images found here agree with those demonstrated by Majumdar et al. in (95) and the NMSE in the MWF map follows the same trend, even with actual CS CPMG acquired data. Thus the original assumption that if the series of individual T2-weighted echo images can be reconstructed accurately, then the MWF map generated from the data will be accurate as well is valid. The study presented here falls under the current techniques of generating parametric maps in two steps, that is, the T2-weighted images are reconstructed first (reconstruction step) and then fitted to find the parameter of interest (estimation step). Because each of the steps introduces errors, one sees less error in the T2-weighted images than in the MWF maps. The former is corrupted only by reconstruction error but the latter is further corrupted by estimation error. One way to reduce these errors is to pose the parametric map estimation as a single step problem, i.e. directly estimate the map from the \(k\)-space samples without the intermediate step of image reconstruction. This is a non-linear least squares problem. When coupled with CS-based sparsity techniques, this becomes an \(l_1\)-regularized non-linear least squares problem. There had been no known solution to the said problem until recently. Even the recent algorithms that exist are only applicable on explicit matrices and not on fast operators like Fourier transforms or wavelet transforms. In the future, as techniques in non-linear CS mature, solving the parametric maps directly from the measured samples may be a possibility.
2.4 Conclusions

The results here show that accurate reconstruction of the T2-weighted echo images from CS accelerated CPMG sequence is possible and that the quality of the MWF maps generated are excellent as well, as long as the acceleration factor is less than two for practical SNR ranges.

2.5 A 3D Extension

After showing that CS can accelerate 2D CPMG acquisition for MWI with good results using a randomized Cartesian sampling scheme, it is only natural to extend the method. The 2D sequence used can be extended into a multi-slice acquisition; however, this suffers from magnetization transfer (MT) effect as the slice selective pulses act like off-resonance saturation pulses and the additional MT contrast interferes with accurate T2 quantitation (96). Therefore, I extended the method to a 3D case to improve acquisition efficiency. With the intrinsically higher SNR efficiency per acquisition time, a larger volume could be covered while retaining similar SNR to the 2D setup. The change to 3D sequence also allows sparse sampling in two phase encode dimensions, translating to a much better sampling scheme while still using a Cartesian acquisition scheme. As well, the additional dimension gives even more correlation for the group-sparsity algorithm to work with. However, the requirement for phase cycling remains, and is even more important in vivo due to $B_1$ field inhomogeneity. A 3D CS CPMG sequence would have the potential to improve image quality by allowing the use of phase cycling with minimal SNR loss when compared to a single signal average. Presently, we do not have the necessary SNR to utilize 3D CS CPMG in vivo. There are other technical challenges as well with the 3D CPMG sequence used, such as the non-selective refocusing portion. Because the spinal cord is
small, most of the body would lie outside of any reasonable FOV and significant aliasing could result if the slice selection profile of the excitation pulse is poor. These could be suppressed by the use of saturation slices (spatially selective 90° saturation pulse applied before the excitation pulse); however, the use of saturation slices introduces MT effect. Therefore, my investigation of the use of CS with 3D CPMG for MWI were limited to \textit{ex vivo} only.

### 2.5.1 Methods

CS 3D CPMG experiments were performed using the same experimental setup and basic parameters as outlined in the previous section. Data was acquired from an excised C4/5 rat cervical spinal cord samples that underwent dorsal column transection injury at the C5 level. The following combination of acceleration factor and averages were used: 3× accelerated dataset at 1 and 2 averages (phase cycled), 2× accelerated dataset at 1 and 2 average, and 1.5× accelerated dataset at 1 average. Fully sampled data with no averages were also acquired as a reference. The sampling scheme used is illustrated in Figure 2.9. All echoes were sampled differently (that is, while each sampling scheme was generated using the same probability density function, they are unique and different from each other) and reconstructed together. Daubechies 8 wavelet at 3 levels of decomposition was used as the sparsifying transform. Group-sparse reconstruction was performed using SPGL1. Myelin water analysis was performed as outlined in §2.2.6. Non-negative least square analysis was used to calculate the T2 distribution for each voxel. Myelin water fraction maps were generated by dividing the integral from 6.738–20 ms range by the total integral of the T2 distribution.
2.5.2 Results and Discussion

Selected results are shown in Figure 2.10 and Figure 2.11. The fully sampled dataset produced excellent MWF map in all slices with the exception of the central slice being corrupted with zipper artifact, shown in Figure 2.12, because of the through slab field inhomogeneity and large volume coverage, the crushers are no longer sufficiently large to suppress the spurious FID signal. In some cases, the zipper artifact can be avoided by using careful slab positioning to place the zipper outside of the region. However, this may be difficult with small FOV and when motion artifact restricts the orientation of phase encode directions. The CS reconstructed echo images were also highly accurate for both datasets. There was a large quality drop from 2× to 3× accelerated scan, resulting in MWF maps of the 2× accelerated scan being significantly better in quality than 3× accelerated scans at 1 or 2 averages, despite taking less time than the latter. This suggests that the issue is related to CS reconstruction artifact, not SNR. Likewise, there was little improvement in data quality in the 3× accelerated dataset from averaging. MWF from various regions of the data shown in Figure 2.11 are displayed in Table 2.1. Overall, there were no large variations in MWF value in WM with the exception of the fasciculus gracilis at 3× acceleration.
This is likely due to the lack of edge definition, resulting in a partial volume like effect. The observed variations in GM are unsurprising because MWF are low in GM to start with. There is an oddly elevated MWF reading in the CST at 1.5×.

![Comparison of 1st and 8th echo images at 1 average (slice 6 of 16)](image)

Figure 2.10. **Comparison of 1st and 8th echo images at 1 average (slice 6 of 16).** The CS reconstructed echo images were highly accurate up to 2× acceleration. The CST, *fasciculus gracilis*, and *fasciculus cuneatus* in the dorsal column were clearly delineated in all images. Signal region is 4 mm across in diameter (cropped).
Figure 2.11. Comparison of MWF maps at different accelerations and averages (slice 4 of 16). There was a large drop in MWF map quality at 3× acceleration.

Figure 2.12. First echo images of the central slice. Data acquired at a single average without phase cycling suffered from spurious FID signal in the central slice. Each pixel is 100 μm × 100 μm.
Table 2.1. MWF of various regions of interest. MWF is calculated for the fasciculus gracilis, corticospinal tract, the entire dorsal column, ventral white matter, and gray matter. Bracket denotes percentage difference from the fully sampled reference MWF map (first line). MWF is elevated compare to literature value due to sample dehydration over long periods of fixation. Errors shown are standard deviation.

<table>
<thead>
<tr>
<th>Acceleration factor</th>
<th>Number of Averages</th>
<th>MWF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gracilis</td>
</tr>
<tr>
<td>1x</td>
<td>1</td>
<td>0.24±0.06</td>
</tr>
<tr>
<td>1.5x</td>
<td>1</td>
<td>0.25±0.03 (4±1%)</td>
</tr>
<tr>
<td>2x</td>
<td>1</td>
<td>0.25±0.07 (2±1%)</td>
</tr>
<tr>
<td>2x</td>
<td>2</td>
<td>0.25±0.04 (2±1%)</td>
</tr>
<tr>
<td>3x</td>
<td>1</td>
<td>0.28±0.06 (15±5%)</td>
</tr>
<tr>
<td>3x</td>
<td>2</td>
<td>0.28±0.04 (13±4%)</td>
</tr>
</tbody>
</table>

In modifying the 3D CPMG sequence for undersampling, changes were made to the phase encoding scheme. While a single phase encode gradient pulse was used to encode all echoes in the fully sampled data, phase encode of opposite polarity around the echo signal were used to allow each echo to be phase encoded differently in the undersampled data, which is less than optimal (92). By encoding each echo individually, spurious FID signal generated by imperfect refocusing pulse becomes phase encoded and is distributed throughout the image. This means that the FID artifact is no longer easily identifiable in the image nor avoidable by careful placement. Thus, the overall data quality is reduced. Unfortunately, it is part of the chosen Cartesian undersampling scheme (later investigation presented in §2.6 shows that it may not be necessary). Fortunately, it can still be removed by phase cycling. The undersampling scheme used is easy to implement in most multi-echo techniques on any scanner. As with the 2D version, a non-Cartesian sampling scheme coupled with better probability density function will likely improve image quality further.
In *in vivo* acquisition, where aliasing requires an increase in FOV, 2× accelerated scans have the potential to reduce a physiologically challenging acquisition time to a reasonable level. However, at the present, we do not have the SNR to spare for performing 3D CS CPMG *in vivo*. As is, we are already at the minimum SNR requirement *in vivo*, and any CS attempt would degrade the data quality under the threshold for meaningful T2 quantitation by NNLS. $B_1$ inhomogeneity is also a concern. Because the spinal cord is small relative to the body and oriented in the head/foot direction, aliasing is an issue when scanning *in vivo*. In the axial plane, this can be dealt with by careful positioning of the animal, as shown in Figure 2.13. Aliasing in the ventral/dorsal direction is limited by the use of a surface coil. Aliasing in the left/right direction does not interfere with the region of interest because of the shape of the body. Unfortunately, the poor slab selection profile of the 90° RF pulse means that a large volume in the rostral/caudal direction would be corrupted by aliasing. To achieve a better profile would have exceeded RF capability of the experimental setup or compromised the sequence’s timing. Alternatively, I could increase the coverage beyond the signal region, but we are already at the lower end of resolution and the upper end of acquisition time. As mentioned before, the use of saturation slices introduces undesirable MT effects. The inhomogeneous field outside the imaging volume also makes the use of saturation slices ineffective.
2.5.3 Conclusions

3D CS multi-echo CPMG with group-sparse reconstruction is a promising approach for increasing acquisition efficiency in myelin water mapping for time constrained experiments. Impact of 3D CS on MWF map quality was found to be minimal as long as the acceleration factor is kept at two or below, which is an improvement over the 2D case presented earlier. While the technique is currently useful for improving the throughput of \textit{ex vivo} experiments, the higher power requirements and the additional field inhomogeneity, combined with the non-selective nature of the refocusing pulse, makes its application difficult \textit{in vivo}.

2.6 A Brief Investigation into Different Echo Sampling and Reconstruction

Thus far, all the image reconstruction presented in this chapter relies on using all of the available information in one step; that is, all the available slices and echoes are reconstructed together. Sampling is also designed to be incoherent in all possible directions. In the 2D case, this not only

\textbf{Figure 2.13. Aliasing in the axial plane.} The solid black outline shows the extent of the animal in the axial plane; the grayscale area shows the signal area using a surface coil setup; and the red outline shows the aliasing in the left/right direction.
improves the accuracy of the T2-weighted echo images, but also the accuracy of the generated MWF maps. However, for the 3D case, this may not be true. In the experiments presented in this section, the reconstruction with and without group-sparsity in the echo direction was tested, as well as different echo sampling methods were used by running simulated CS experiments using several fully sampled \textit{ex vivo} datasets and one \textit{in vivo} dataset. When performing reconstruction without utilizing group-sparsity in the echo direction, 3D data from each echo is reconstructed individually, while with group-sparsity, all 3D data from all echoes are reconstructed simultaneously.

2.6.1 Methods

Five \textit{ex vivo} paraformaldehyde fixed cervical spinal cord samples excised from female Sprague-Dawley rat were scanned using the same experimental set-up as outlined in §2.2.2. Fully sampled 3D CPMG experiments were carried out with the following parameters: 128 × 128 × 16 matrix, TE/TR = 1500/6.738 ms, 6.738 ms echo spacing, 32 echoes, 1.28 × 1.28 × 1.6 cm FOV, 2 averages. One set of \textit{in vivo} data was also included. Data for the \textit{in vivo} case were acquired with a surface receive coil from the cervical spine, using the same parameters. As with the 2D case, undersampled data was generated from fully sampled 3D data by discarding PE lines. The PE sampling scheme used is illustrated in Figure 2.9. The readout lines were fully sampled.

Two types of echo sampling were used. The first type of sampling scheme follows from the 2D case, where each echo was sampled differently; that is, while each echo shares the same probability density, they were generated individually and therefore different. The second type of sampling scheme used identical echo sampling; that is, each echo was sampled exactly the same
as the first. The goal was to see if the different echo-sampling scheme has an effect on MWF generation. Individual echo reconstruction is of special interest, because it allows parallel reconstruction and lowers the memory requirement. Compared to the single threaded full reconstruction with all the echoes, individual echo reconstruction with parallel threads significantly reduced computational time.

Data sets were reconstructed two ways, once as four-dimensional data set including all echoes, and again as individual 3D reconstructions at each echo. Two levels of acceleration were tested: 1.5× and 2×. Once again, SPGL1 was used for group-sparse reconstruction and in-house software used to generate MWF maps.

### 2.6.2 Results and Discussion

Results of the 1.5× accelerated data are shown in Table 2.2. Unsurprisingly, the least amount of error results from datasets where the echoes were reconstructed together; however, more interesting is the result of different echo sampling. Unlike 2D CS CPMG, randomized echo sampling did not achieve the most accurate MWF map. Looking at the NMSE of the T2 echo images, the best image quality was achieved by utilizing both the group-sparsity and randomized sampling in the echo direction. However, when looking at the NMSE in the MWF maps, generated from all tested datasets, identically sampled echoes reconstructed together provided the best result. Even in the cases where echoes were reconstructed individually, identical echo sampling appeared to have performed equally as well as random sampling. It seems that choosing between random and non-random sampling is a compromised between reducing aliasing artifacts and promoting sparsity, and recovering accurate T2 decay curve. One could
speculate that, for 3D CS CPMG, the extra correlation from the additional phase encode dimension negates some of the need for correlation in the echo direction. Because there is more space to spread out the aliasing artifacts to reduce their magnitude in 3D, the need to have a consistent pattern of aliasing from echo to echo outweighs the need to reduce aliasing through random sampling.

Results of 2× accelerated data are shown in Table 2.3. It followed closely the trends established in the 1.5× accelerated cases with a few exceptions. While the results in the 2× case were less clear cut, one can reasonably expect the same echo sampling to perform better in actual CS experiments, because of the potential reduction in artifacts resulting from the removal of PE gradients between echoes (instead, a single PE gradient pulse encodes all the echoes) (21). It remains to be seen whether the same result carries into actual CS experiments.

Table 2.2. NMSE of 1.5× Accelerated Data. NMSE were calculated from ROI that covered the entire spinal cord sample in both the T2-weighted echo images and MWF maps.

<table>
<thead>
<tr>
<th>Same Echo Sampling</th>
<th>Ind Echo Recon</th>
<th>Ex Vivo 1</th>
<th>Ex Vivo 2</th>
<th>Ex Vivo 3</th>
<th>Ex Vivo 4</th>
<th>Ex Vivo 5</th>
<th>In Vivo</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>T2 MWF</td>
<td>T2 MWF</td>
<td>T2 MWF</td>
<td>T2 MWF</td>
<td>T2 MWF</td>
<td>T2 MWF</td>
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<tr>
<td>Yes</td>
<td>No</td>
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<td>0.01418 0.19369</td>
<td>0.01398 0.26177</td>
<td>0.016057 0.28967</td>
<td>0.02428 0.30381</td>
<td>0.03225 0.36229</td>
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<tr>
<td>No</td>
<td></td>
<td>0.01750 0.30207</td>
<td>0.01309 0.19154</td>
<td>0.01358 0.27903</td>
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<td>0.02178 0.31256</td>
<td>0.02913 0.42473</td>
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<tr>
<td>Yes</td>
<td>Yes</td>
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<td>0.01507 0.22713</td>
<td>0.01536 0.32351</td>
<td>0.018645 0.33689</td>
<td>0.02519 0.34531</td>
<td>0.03699 0.44905</td>
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<td>0.01536 0.32775</td>
<td>0.018645 0.36615</td>
<td>0.02519 0.37326</td>
<td>0.03699 0.52845</td>
</tr>
</tbody>
</table>

Table 2.3. NMSE of 2× Accelerated Data. NMSE were calculated from ROI that covered the entire spinal cord sample in both the T2-weighted echo images and MWF maps.

<table>
<thead>
<tr>
<th>Same Echo Sampling</th>
<th>Ind Echo Recon</th>
<th>Ex Vivo 1</th>
<th>Ex Vivo 2</th>
<th>Ex Vivo 3</th>
<th>Ex Vivo 4</th>
<th>Ex Vivo 5</th>
<th>In Vivo</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td>T2 MWF</td>
<td>T2 MWF</td>
<td>T2 MWF</td>
<td>T2 MWF</td>
<td>T2 MWF</td>
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</tr>
<tr>
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<td>No</td>
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<td>0.02159 0.27490</td>
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<td>0.036729 0.67453</td>
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<tr>
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<td>0.03340 0.67140</td>
<td>0.036729 0.61130</td>
<td>0.04712 0.48535</td>
<td>0.06617 0.75190</td>
</tr>
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</table>
2.6.3 Conclusions

With 3D CS CPMG for MWI, there is little need for different sampling scheme between echoes. Unlike in 2D CS CPMG, more accurate MWF maps can be generated by keeping the same sampling scheme from echo to echo. This suggests that there is a limit to utilizing group-sparsity in the echo direction.
Chapter 3: Gray Matter Oddity in Spinal Cord Tissue during Aldehyde Fixation

It was mentioned that one of the major goals of this thesis was to investigate the use of myelin water imaging (MWI) to quantify myelin content in the presence of myelin debris by correlating MR-derived myelin measurement to transmission electron microscopy (TEM) derived myelin measurements. This requires finding a fixation protocol that works well for both high-resolution TEM and MR imaging. Along the way, we came across a couple of interesting phenomena that warrant documenting. However, due to time constraints, only a small set of data from each case was acquired. One of the most interesting phenomena is the loss of contrast in MWF map due to elevated gray matter (GM) MWF immediately after perfusion fixation, and the subsequent gradual decrease in GM MWF to its normally observed values over time in post fixation. These sets of data are presented and speculated upon in this chapter as pilots for future investigations; therefore, the usual chapter structure was abandoned in favour of a more narrative style.

3.1 Background
For the longest time, the go-to fixation protocol for our ex vivo scan had been the same: the rats were anesthetized and perfused intracardially with phosphate buffered saline (PBS) for 3 minutes, followed by 4% paraformaldehyde (PF) in 0.1M sodium phosphate buffer at a pH of 7.4 for 30 minutes. The spinal cords were harvested and kept in the same fixative overnight for post-fixation before MR experiments. This protocol worked well for most immunohistology studies and produced consistent myelin water fraction (MWF) maps even weeks after the initial scan.
However, when processing these samples for high-resolution TEM, they turned out to be inadequately fixed (97). The inadequate fixation is not only difficult to process but, also produced artifact-ridden samples incapable of supporting high-resolution TEM imaging. The issue is also compounded by the lack of tissue integrity in the injured samples, which in turn put an even higher demand on good tissue fixation. An example of the lack of definition with the original fixation protocol is illustrated in Figure 3.1.

![Figure 3.1](image)

Figure 3.1. Example of low-quality TEM data. The low image quality of the myelin debris is a result of poor sample integrity due to injury-induced degeneration and inadequate fixation.

The first stage to fixing this problem was to add glutaraldehyde (GD) into the mixture. While both PF and GD form methylene bridges that cross-link protein to stabilize biological samples, they serve slightly different roles. PF penetrates quickly due to its smaller molecular size, but the
larger GD is a better cross-linker with an extra aldehyde group. The extra aldehyde group not only allows it to form more links, but when combined with its larger size gives GD extended reach and allows it to bind protein molecules farther apart. All these properties make it well suited for TEM fixation. Thus, the 4% PF fixative was replaced by a modified Karnovsky’s solution (4% PF and 1% GD) in hope of better tissue preservation (98). In addition, instead of post-fixing for one hour in 2% GD after the MR experiment, GD fixation was done before the MR experiment and lengthened to overnight. However, these changes did not improve the TEM data quality.

At this point, the culprit seemed to be the overnight post-fixation stage, as it was now the only major modification made in concession of the MR experiments. This lengthy post-fixation stage delays the lipid fixation stage with osmium tetroxide and potassium ferrocyanide from being performed at 90 minutes to over twelve hours after excision. The logical action then was to try to shorten this delay in lipid fixation by starting the MR experiment after only one hour of post-fixation in 2% GD.

A flow chart illustrating the evolution in the fixation procedure is shown below, in Figure 3.2.
The process of arriving at the final protocol used in Chapter 6 is shown. The process was guided by existing literature but each method was tested experimentally. Unrealistic outcomes of all decision symbols have been omitted. The red decision symbol marks when the gray matter oddity occurred.

### 3.2 The Experiments

All MRI experiments were carried out on a 7 T animal scanner (Bruker, Germany) using a single slice multi-echo CPMG sequence with a 13 mm inner-diameter, 25 mm long five-turn solenoid coil and TR/TE = 1500/6.738 ms, 6.738 ms echo spacing, 32 echoes, 256 × 256 matrix size. Various slice thickness and FOVs were used. All slices were prescribed at 5 mm cranial to...
injury. Fifteen mm long sections of spinal cord centered on C5 were excised from normal and post C5 dorsal column transection injured Sprague-Dawley rats at 3 and 8 weeks post-injury time point. All NNLS fitting were done with IGOR Pro (WaveMetrics, Lake Oswego, OR). Once the spinal cord sample was transferred into the sample holder, post-fixation was considered to have stopped as the fixative to sample volume ratio was too small (<< 15).

Figure 3.3 shows the MWF map generated with only 90 minutes of post-fixation. All samples showed elevated MWF reading in the GM, with the effect being most pronounced in normal and 8 weeks post-injury samples. In the normal and 8 weeks post-injury samples, the contrast in the MWF map was almost completely lost. There was abnormally high MWF in the corticospinal tract (CST) in many of the samples, as well as in part of the fasciculus gracilis in the injured cord samples. Typically, increased MWF in the CST and decreased MWF in the fasciculus gracilis are seen post injury (74). When looking at the T2 distribution, the GM distribution showed a large myelin water component but was otherwise relatively normal looking, with the usual T2 values of myelin and intra/extracellular water (Figure 3.6). However, the CST showed a very large myelin water peak with T2 value at the lower limit of the regularized T2 distribution (Figure 3.3). The tissue fixation procedure was reviewed and fresh fixatives were made and remade. Multiple samples later, I was convinced that this effect is real. Furthermore, when one of the normal samples was rescanned after overnight post-fixation (the sample was returned to 50 mL of fixative at 4°C, exceeding the best practice of 50:1 of fixative to tissue volume ratio), the expected contrast was recovered (including in the CST, see Figure 3.4). This prompted us to try a dynamic study, where I scanned the samples at fixed intervals through several hours of post-
fixation. Results are shown in Figure 3.5 and Figure 3.6. In both cases, the T2 distribution in white matter (WM) remained stable while MWF decreased over time in GM.

![Figure 3.3. MWF map generated after one-hour post-fixation.](image)

Figure 3.3. MWF map generated after one-hour post-fixation. Figure shows three normal control samples, three 3 weeks post injury, and one 8 weeks post injury samples. Images were acquired at 70 µm by 70 µm in-plane resolution with 0.5 mm slice thickness, with the exception of the 8 weeks post-injury sample, which was acquired at 100 µm by 100 µm in-plane resolution with 1 mm slice thickness. Slice was prescribed at 5 mm cranial to injury. Besides the oddity with gray matter, the corticospinal tract showed abnormally high MWF in many of the samples. The injured cord also showed abnormally high MWF in part of the the gracilis. Vertical bar shows the scaling of the colour map.
Figure 3.4. MWF map after overnight fixation. The normal control cord sample highlighted in yellow in Figure 3.3 was re-immersed in a large volume of GD fixative overnight before rescanning, resulting in the expected MWF in GM. The overnight fixed cord was scanned at 100 µm by 100 µm in-plane resolution with 1 mm slice thickness.

Figure 3.5. MWF maps and T2 distributions after additional post-fixation. The 8 weeks post-injury cord sample highlighted in red in Figure 3.3 was re-immersed in a large volume of GD fixative for 30 minutes and rescanned (middle), afterward it was returned for another hour of fixation before the final scan (bottom). T2 distributions in GM after 1 and 2.5 hours of post-fixation are shown on the right. Vertical bar shows the colour map.
Figure 3.6. **MWF map of a normal control cord sample at various post-fixation times.** Images were acquired at 100 µm in-plane resolution with 1 mm slice thickness. Every hour, approximately 2 mL out of 3 mL total volume of fixative in the sample holder was refreshed. This requires repositioning the sample so the slices shown are not implicitly coregistered. MWF in GM decreased with fixation time. However, MWF of the CST was still elevated at the 4-hour time point, which may be due to the small volume of fixative replaced. Vertical bar shows the colour map.

For additional information, a cord sample was excised from an uninjured rat that did not undergo perfusion fixation. The cord sample was then scanned in phosphate buffered saline at 1 hour and 1.5 hours after excision. It then underwent 30 minutes of immersion fixation in the same fixative in between 30 minute scans until 6.5 hours after excision. The result is shown in Figure 3.7. At the beginning, the MWF of the unfixed cord sample matched the *in vivo* value we typically get (~0.2). No change was observed in MWF after the cord was left unfixed for 2.5 hours. This suggests that for at least a couple of hours, MWF was not sensitive to any of the changes resulting from post-mortem decay, such as the autolysis of protein and lipids (99,100). During immersion fixation, the MWF of WM gradually increased to what we typically see in fixed tissue (~0.4). The T2 of the intra/extracellular component decreased, and the myelin water component
in GM disappeared. While the GM MWF in both cases were what we expected, their low value is another phenomenon that we do not yet understand. MWF in GM is consistently underestimated in all the data, whether in vivo or ex vivo. Nevertheless, based on the immersion fixation data, the GM oddity appears to occur only with perfusion fixation.

**Figure 3.7. MWF map of a normal control cord sample at various immersion fixation times.** Images were acquired at 100 µm by 100 µm in-plane resolution with 1 mm slice thickness. After the first two time points (sample stored in phosphate buffered saline and is unfixed), the sample was fixed with PF + GD. Each time point is half an hour of immersion in a large volume of fixative followed by half an hour of imaging (in 3 mL of fixation sample holder). Note the increase in MWF in WM as tissue is fixed. This matches our experience. The GM artifact does not seem to show up here.
The result demonstrated that the effect was most prominent in the normal control and 8 weeks post DC Tx injury samples and did not seem to affect 3 weeks post-injury samples to the same extent. In addition, unfixed samples that underwent immersion fixation using the same fixative solution did not exhibit the same problem. Perhaps the problem depends on perfusion and tissue structure, especial with GM being highly vascularized, which could explain why the phenomenon is isolated to the GM after perfusion fixation. Vasoconstriction from perfusion fixation may reduce the intra/extracellular water content. Macromolecular flushing and changing membrane permeability are two other possible mechanisms. However, more studies are needed to explain these effects fully. After observing this phenomenon, we briefly investigated the possibility of scanning heavy metal fixed spinal cord samples in MR. The result is presented in Appendix B. For the experiments presented throughout the rest of this thesis, we resorted to dividing the cord into pairs of contiguous slices that produced individual TEM and MR samples that can be fixed differently. The process will be described in more detail in the next chapter.
Chapter 4: Effect of Osmolarity of Fixative on Myelin Water Imaging

Myelin content is an important marker for central nervous system pathology. Quantitative T2 based myelin water imaging has been shown to measure myelin content in normal and diseased brain and spinal cord tissues (45, 47). Because myelin is difficult to image directly with MR due to very short T2 relaxation times (101), this technique focuses on indirect measurement of myelin content by probing the properties of the surrounding water. Therefore, it is important to understand how changes in tissue morphology affect the water environments. Rat models are widely used to study spinal-cord injuries and associated repair therapies; however, due to the challenges in acquiring 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. Still, fixation procedure may affect tissue properties and, subsequently, the measured myelin water fraction (MWF). This study looks at the effect of phosphate buffer (PB) concentration on the shape of T2 distribution and how myelin water fraction (MWF) calculations are affected.

The tissue shrinkage/dehydration effect of aldehyde fixative is well known (102, 103) and there have been studies showing that at least part of it is caused by osmotic gradients (104). Several things could happen as the PB concentration is varied. Generally, increased PB concentration could pull water out of any compartment that is impermeable to PB ions (HPO$_4^{2-}$, H$_2$PO$_4^+$, H$_3$PO$_4^-$, etc.), and thus membrane permeability needs to be considered. However, because the ends of the dorsal column in the samples were severed, the extracellular spaces are likely to be contiguous with the fixative. For the same reason, this also means that most of the axons in the normal control samples are now open on both ends. Therefore, in this model, change in PB
concentration would have no effect on these unsealed water compartments. On the other hand, due to the short length of myelin sheaths (~30 µm, up to 1 mm (27)), and their general impermeability to phosphate ions, the myelin water compartment stays sealed. Thus, in samples with intact myelin sheaths, we expect to see the osmotic gradient preferentially removing myelin water. These potential changes could also affect the T2 of each compartment, which in turn could be influenced directly by the PB concentration.

This study was designed as an addition to the myelin debris study that will be presented later in Chapter 6. Compared to previous studies that did not use high-resolution TEM, the myelin debris study used only 4 mm of the cervical spinal cord located between 2 mm and 5 mm cranial to C5. By utilizing the rest of the available length of the cord, four additional samples could be created for the osmolarity study from each of the six normal control spinal cord samples, for a total of 24. The pairs are comparable because there is no intermingling of neuronal cell bodies in the tracts at the cervical levels that we are interested in. From previous studies (6,47,74), it is known that the MWF from 10 mm caudal to 5 mm cranial of C5 in the dorsal column of uninjured cord was consistent. The interfaces of the four pairs of samples fall within this range, and thus are comparable.

4.1 Methods

Uninjured ex vivo cervical cord samples were obtained from Sprague-Dawley rats as outlined in §6.1.1. For each rat, the cervical spinal cord cut into eight sections alternating between 3 mm and 1 mm with adjacent 3 mm and 1 mm section constituting a pair (see Figure 4.1). Each pair was fixed in 2% glutaraldehyde solutions of various PB concentrations (0.35 M, 0.27 M, 0.21 M, 0.16 M, 0.10 M, and 0.05 M).
0.14 M, 0.10 M, 0.07 M, 0.046 M, 0.023 M, and 0 M randomly assigned). All 1 mm MRI sections were post-fixed overnight before scanning. All 3 mm TEM sections were post-fixed overnight as well, with the exception of the 0.10 M samples, which were only post-fixed in 2% glutaraldehyde for an hour. This was done to ensure that the 0.10 M sections, which are also the normal control sections for the myelin debris study, receive the best TEM fixation possible.

![Diagram](image.png)

**Figure 4.1. Sectioning of cervical spinal cord for osmolarity study.** The cervical spinal cord cut into eight sections alternating between 3 mm and 1 mm with adjacent 3 mm and 1 mm section constituting a pair.

MR experiments were carried out on a 7T preclinical scanner (Bruker, Germany) using a five-turn, 13 mm inner-diameter solenoid coil. Quantitative T2 data was 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, 6.738 ms echo spacing, 32 echoes, 1.79 cm FOV, 1 mm slice, NA = 12, 70 μm in-plane resolution) (21). CPMG data was processed using a non-negative least square analysis technique (77). Geometric mean T2 (GMT2, defined in Eq. 1.85) 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 6.738 ms – 20 ms, corresponding to previous studies (4,74), and the second one with the upper cutoff equal to the geometric mean of the intra/extracellular (I/E) water and MW T2 values, determined from ROI analyses of the *fasciculus gracilis*.

TEM fixation and image acquisition were carried out as described in §6.1.3. TEM images at 16,000× magnification (5.83 µm × 5.83 µm at 2048 × 2048) were used to quantify the myelin content. The full 16-bit grayscale was normalized to between 0 and 1 by mapping the 0.1 to 99.9 percentile pixels linearly. Histogram normalization was performed on these images to produce a flat histogram using 64 bins (see the insets of Figure 4.2). Myelin area was manually segmented and the segmented area thresholded to exclude large, watery spaces as illustrated in Figure 4.2 (for more details on these spaces, see Chapter 6). Myelin fraction was calculated as the myelin area minus the large watery spaces and divided by the total area. That is, the TEM-derived myelin fraction is an area fraction of compacted myelin.
Figure 4.2. TEM data processing. From left to right, the TEM image undergoes histogram normalization, manual segmentation, and simple thresholding. The resulting myelin fraction is the area fraction of the rightmost image. Insets show image histograms. The scale bar in red shows 1 µm.

4.2 Results and Discussion

Changes in PB concentration created a large change in T2, which warrants the myelin water cut-off to be moved (Figure 4.3). Figure 4.4 shows the dependence of GMT2 of both the fixative solution and the WM of fasciculus gracilis on PB concentration. Figure 4.5 shows the dependence of MWF on PB concentration before correction (MWF range 6.738 – 20 ms) and after correction (MWF range 6.738 – 13 ms). The fasciculus gracilis was chosen for the analyses because there is little intermingling of neuronal cell bodies at the cervical level and it provides a consistent MWF through all sections studied here. The results indicated a large increase in GMT2 of the fixative with increasing PB concentration. The fasciculus gracilis followed a similar increasing trend, albeit at a much smaller rate. There was a curiously large initial drop in GMT2 in the fixative from 0 M (unbuffered) to 0.023 M that was not reflected in the fasciculus.
gracilis. The unbuffered fixative solution also showed greater variability in MWF than the buffered fixative solution.

![Graph showing T2 distribution in fasciculus gracilis at two different PB concentrations.](image)

**Figure 4.3. Myelin water cut-off correction.** The graph shows an example T2 distribution in the *fasciculus gracilis* at two different PB concentrations. The 20 ms cut-off used at 0.10 M PB needed to be adjusted to generate the corrected MWF at 0.07 M PB.
Figure 4.4. Dependence of T2 on PB concentration. GMT2 in both the *fasciculus gracilis* and the fixative solution indicate an increasing trend.

Figure 4.5. MWF dependence on PB concentration. Uncorrected MWF shows a decreasing trend with buffer concentration as previously reported (4); however, when the MW range is corrected for the T2 dependence on PB concentration, this trend disappears.
As the GMT2 shortened with decreasing concentrations of PB, both the I/E and MW water peaks were pushed to shorter T2 times and became broader; as a result, the fixed cut-off that worked well at the standard concentration of 0.21 M started to classify part of the I/E peak as MW, and artificially inflated the reported MWF. One potential way to correct for this, especially when the T2 peaks are well separated, is to detect each peak individually. However, this runs into problems when the peaks overlap, so to correct for this effect, MWF was generated with the upper cutoff reduced to the mean T2 of I/E and MW water peaks of the *fasciculus gracilis*. With this corrective step in place, the MWF reading appeared to return to “historical” levels, with the exception of MWF readings taken in the absence of PB. As the MW water peak moves to shorter and shorter T2, eventually, its T2 becomes less than twice the echo spacing and MW peak location can no longer be reliably determined. However, even in the case where the MW T2 are at the lower limit of a regularized distribution, reliable MWF can be calculated (105). The consistency of MWF over the range of PB reflects this robustness.

Due to unfortunate complications (sudden departure of a collaborator due to illness, leaving no records of the samples, and time constraint), only nine data points were obtained for the TEM result, which are shown in Figure 4.6. Much like the MWF results, the TEM measurements clustered around the same range; however, there is a slight upward trend in TEM measured myelin fraction versus PB concentration, although not significant. The data suggests that TEM myelin fraction measurement does not depend on the PB concentration used in the post-fix stage. This is unsurprising because we know that one way to reduce MWF inflated by aldehyde fixation is to wash the sample successively in PBS before scanning. The TEM myelin fraction is
therefore more likely to depend on the rest of the fixation stages then the glutaraldehyde post-fixation stage. Irreversible changes may need longer than an overnight fixation to manifest itself.

Figure 4.6. TEM measured myelin fraction versus PB Concentration. TEM-derived myelin fraction vs PB concentration shows a slight upward trend; however, it is not significant. Because the data points were derived from only three animals, the p-value does not fall below a Bonferroni corrected significance level at 0.0167 ($R^2 = 0.4832, p=0.038$).

The polymerization rate of glutaraldehyde and tissue integrity is dependent on the pH of the fixative solution (106). Typically, TEM samples are fixed at physiological pH. Because the size of the sample was very small compared to the amount of fixative used, it would have little effect on the pH throughout the range of PB concentration. Instead, the role of the buffer is to keep the fixative solution at a stable pH as glutaraldehyde slowly oxidizes to form glutaric acid (it will also polymerize to form cyclic and oligomeric compounds) (107). If the low end of PB concentration (0.023 M) was able to keep the fixative stable during the experimental time frame,
it may explain the highly variable GMT2 of the fixative and MWF of the *fasciculus gracilis*. It may also help to explain the discrepancy in GMT2 within the cord between the unbuffered case and the standard practice of 0.1 M PB, despite sharing similar GMT2 of the fixative. The varying concentration of PB not only changes the osmolarity, but introduces other complex factors to consider.

It was speculated earlier that the osmotic pressure might preferentially remove water from the myelin water space due to the general impermeability of the myelin membrane to phosphate ions. Because no MWF change was seen over the entire range of PB concentration, this is likely not the case. Instead, the results suggest that PB had equal access to both compartments and helps explain the change in T2 of both compartments, unless the effect was counteracted by some other process, such as proton exchange.

The proton donating and proton accepting nature of PB needs to be considered here. By adding PB to the system, a host of new proton transfer pathways were introduced (108). Through these pathways, PB can facilitate indirect proton exchange between water molecules. Potentially, this leads to an increase in exchange between the water compartments, as had been observed in NMR experiments of bovine cartilage (109). Based on this, it may not be surprising to see that the changes in the location of the MW and I/E peaks shown in Figure 4.3 look suspiciously like the effect of inter-compartmental water exchange between the MW and I/E water compartments. However, the fact that the corrected MWF remained stable over the entire range of PB concentration contradicts the predicted decrease in MWF with increased exchange (93,110).
Furthermore, if exchange really were a factor, then the T2 of both components would have moved toward shorter T2 times with increasing PB concentration, which was not the case. The results showed longer I/E water T2 with PB concentration, and no obvious trend in the MW T2 (especially because the MW T2 values are near or at the low end of the distribution, quantitation was unreliable). Lastly, the change in MW and I/E peak location can be explained solely by the overall drop in T2 with decreased PB concentration, as observed in the fixative. It is possible that both mechanisms were involved. For example, if the decrease in MW due to exchange was masked by an increase in MW from the changing osmolarity; however, this is rather unlikely. Because the rate constant increases with PB concentration, the increase in proton exchange is of the second order would result in a fairly large range of exchange rates (108,111). However, the fact that the MWF was stable throughout, as long as the fixative was buffered, suggests that any potential water exchange would be too slow to have any significant effect on the MWF measurements. Nevertheless, varying the PB concentration may provide a convenient and novel method for investigating the much-debated role of water exchange in myelin water imaging.

4.3 Conclusions

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. While the results showed no change in MWF with osmolarity, the amount of PB used had a large impact on the measured T2. There was a steady increase in T2 in both the fixative solution and the spinal cord sample with increasing PB concentration. This not only has an important implication in myelin water imaging, but also for any MR acquisition where PB is involved. The fact that PB is widely used in *ex vivo* scans, especially in the form of phosphate
buffer saline, speaks of the importance of the results. Knowledge of T2 change with PB concentration can be useful in phantom preparations. While the results did not seem to depend on PB facilitated increased in proton exchange, the potential use of PB to alter the proton exchange rate between water compartments may be useful in future studies.
Chapter 5: Myelin Water Imaging in the Presence of Myelin Debris

A wide array of processes occurs after spinal cord injury, including white matter demyelination and proximal and distal Wallerian degeneration. The interruption and demyelination of the axonal tracts ultimately lead to the functional loss seen in spinal cord injuries (112). Therefore, it is important to have a non-invasive technique that is able to track myelin in spinal cords. There are several MR techniques aimed at this, including quantitative T2, diffusion tensor imaging (DTI), and quantitative magnetization transfer. Because myelin is difficult to image directly with MR due to very short T2 relaxation times, these 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. While quantitative T2, DTI, and magnetization transfer are able to generate indirect measures of myelin and axonal density, there is currently no technique capable of distinguishing between intact/functioning myelin and myelin debris (47,52,74,75). Here I use myelin debris to describe myelin sheaths in all stages of degeneration that still retain the unique lipid bilayer structure. A timeline of myelin degeneration can be found in §1.3.4.1. Light microscopy based studies have suggested that MWF measures both intact and myelin and myelin debris in a rat spinal cord injury model.

5.1 Pilot Study Part I

This study focuses on comparing several MR parameters between intact myelin and myelin debris in a rat spinal cord injury model.
5.1.1 Methods

DTI, quantitative T2, and T1 mapping experiments were used to characterize eight excised rat spinal cord samples at 3 weeks following dorsal column transection (DC Tx, as outlined in §1.3.4.1) injury (45). Data was acquired using a four-turn 2 cm inner-diameter solenoid transmit/receive solenoid coil. Slices were acquired at 5 mm cranial to injury with a resolution of 100 μm × 100 μm × 1 mm. DTI data was acquired using a multi-slice spin-echo (SE) based sequence (TR/TE = 1500/21.337 ms, b = 750 s/mm², 6 non-collinear directions using icosahedral encoding scheme (113), 4 slices, 128 × 128 at 1.28 cm FOV, 4 averages); quantitative T2 data was acquired using a single slice multi-echo CPMG sequence (47) (TR/TE = 1500/6.738 ms, 6.738 ms echo spacing, 32 echoes, composite refocusing pulses, descending alternating crushers, 1 mm slice, 128×128 at 2.56 cm FOV, 6 averages); and T1 data was acquired using a series of single slice SE inversion recovery scans (TR/TE = 12000/8 ms, echo train length of 4, TI = 500, 1000, 1500, 2700, 5000, and 10000 ms, 128 × 128 at 1.28 cm FOV, 1 average).

DTI data was processed to generate maps of apparent diffusion coefficient (ADC), transverse diffusivity ($D_{\text{trans}}$), longitudinal diffusivity ($D_{\text{long}}$), and fractional anisotropy (FA) using in-house developed Matlab (Natick, MA) code (114). CPMG data was processed using a non-negative least square analysis technique (77) to generate myelin water fraction (MWF) and geometric mean T2 (GMT2) maps, using IGOR Pro (WaveMetrics, Lake Oswego, OR). MW was crudely estimated by combining the proton density map calculated from the T1 data and the MWF calculated from the T2 data. No field inhomogeneity correction was applied.
Region of interest (ROI) analysis was used to obtain average values of ADC, $D_{\text{trans}}$, $D_{\text{long}}$, FA, MWF, MW, and GMT2 from the injured *fasciculus gracilis* and adjacent uninjured region of white matter (*fasciculus cuneatus*). Statistical significance of the difference between group means was assessed using two-tailed t-test. MR results were qualitatively compared to selected high-resolution optical microscopy and electron microscopy of plastic sections of the injured cords.

5.1.2 Results and Discussion

A summary of the results from the ROI analyses is shown in Table 5.1. DC Tx injury results in damage to *fasciculus gracilis* (ascending sensory tract) cranial to injury (47). The axonal damage is demonstrated by the significant decrease in $D_{\text{long}}$, which correlates well with axon integrity in a variety of injury models (115–117). This is also partly the cause of the observed decay in FA.

Myelin damage is more difficult to assess due to the presence of myelin debris as a result of Wallerian degeneration. Both MWF and MW increased as a result of injury, although differences were not statistically significant. This is consistent with a previous report by Webb in the peripheral nervous system (PNS) (52) suggesting that the amount of myelin water is a measure of both intact myelin and myelin debris. It is somewhat surprising that the average T2 showed a small decrease as a result of injury, as one would expect an increase in T2 of myelin water due to increased volume of the myelin water compartment. However, increase in myelin spacing has not been observed in this these or elsewhere. More likely, this decrease in average T2 may potentially reflect a significant decrease in T2 of intra/extracellular water, judging from the morphological changes observed in electron microscopy images (see Figure 5.1), which would
mitigate a potential increase in T2 of myelin water. The increased $D_{\text{trans}}$ likely reflects morphological changes in WM following axonal degeneration as a result of the injury. In general, $D_{\text{trans}}$ is influenced by several factors, including axon density, and the amount of extracellular water (118). It had also been proposed as an indicator of myelin content (65,66). However, later studies put this in doubt (57,67–69). Likewise, the increased seen in $D_{\text{trans}}$ was not accompanied by a significant reduction in the MWF. Consequently, the increase in $D_{\text{trans}}$ can be attributed to axonal degeneration and the increase in the amount of extracellular water (that replaces the loss in the axons’ intracellular water), including the large watery spaces seen in Figure 5.1. As the axons disintegrate, myelin debris becomes irregular, onion-shaped like structures, no longer aligned in one distinct direction. As a result, the diffusion process becomes more isotropic, which is reflected by a decrease in FA and $D_{\text{long}}$, and increase in $D_{\text{trans}}$. Note that the T2 shown here is shorter than in vivo situations due to the effect of aldehyde fixation (119).

**Table 5.1. Average values, standard deviations, and p-values of various MR parameters.** Bolded parameters and p-values signifies statistically different results at $p < 0.05$ between 3 weeks post injury and control region at 5 mm cranial to injury.

<table>
<thead>
<tr>
<th></th>
<th>Injured</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average ± S.D.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Water</td>
<td>3.08±0.24 × 10^4</td>
<td>3.30±0.24 × 10^4</td>
<td>0.28</td>
</tr>
<tr>
<td>$D_{\text{trans}}$</td>
<td>1.59±0.27 × 10^4</td>
<td>1.28±0.52 × 10^4</td>
<td>0.33</td>
</tr>
<tr>
<td>MWF</td>
<td>0.515±0.066</td>
<td>0.385±0.138</td>
<td>0.14</td>
</tr>
<tr>
<td>T1 (s)</td>
<td>0.705±0.201</td>
<td>0.695±0.222</td>
<td>0.95</td>
</tr>
<tr>
<td>GMT2 (ms)</td>
<td>20.9±1.8</td>
<td>22.7±2.2</td>
<td>0.22</td>
</tr>
<tr>
<td>$D_{\text{long}}$</td>
<td>0.54±0.10</td>
<td>0.78±0.10</td>
<td>0.0026</td>
</tr>
<tr>
<td>ADC</td>
<td>0.37±0.11</td>
<td>0.34±0.07</td>
<td>0.64</td>
</tr>
<tr>
<td>FA</td>
<td>0.45±0.13</td>
<td>0.83±0.06</td>
<td>0.00010</td>
</tr>
</tbody>
</table>
Figure 5.1. Optical microscopy image of injured (left) and uninjured (right) white matter, and electron microscopy image of myelin debris (middle). The myelin membranes in the uninjured white matter show tight packing around the axons. The red arrows point to myelin debris, characterized by increased spacing between lipid bilayers, which is shown more clearly in the electron microscopy image in the middle. The large watery spaces shown in the middle are expected to contribute to the increase in $D_{\text{trans}}$.

5.2 Pilot Study Part II

For this study, we focused on the injured fasciculus gracilis 5 mm cranial to injury at 3 and 8 weeks post-injury, where we expect, from previous findings, that myelin debris is present at 3 weeks and is partially cleared by 8 weeks following injury. In addition to MR experiments, electron microscopy was used for histology imaging. The coil setup had also been upgraded, from a four-turn, 20 mm inner-diameter solenoid to a five-turn 13 mm inner-diameter solenoid. Length increased from 20 mm to 25 mm.
5.2.1 Methods

All MRI experiments were carried out on a 7 T animal scanner (Bruker, Germany). 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 (45). 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 = 6.738/1500 ms, 6.738 ms echo spacing, 32 echoes, 2.56 cm FOV, 1 mm slice, NA = 6, 100 µm in-plane resolution). CPMG data was processed using a non-negative least square analysis technique. Myelin water fraction (MWF) maps were generated by dividing the integral from 6.738–20 ms range by the total integral of the T2 distribution. Region of interest analysis was used to obtain the average values of MWF from the injured fasciculus gracilis, where myelin damage is most prominent in this model (47). Statistical significance of the difference between group means was assessed using two-tailed t-test. T2 relaxation times of the short and long water components were measured in the same region on a voxel-by-voxel basis (121).

From each sample, transmission electron micrographs (TEM) were generated at 10,000× magnification at a resolution of 2048 × 2048 pixels on a Hitachi H7600 transmission electron microscope (Hitachi Ltd., Tokyo Japan) at 5 mm cranial to injury in the fasciculus gracilis. These were stitched together to form a thin column. Lines were drawn at 500-pixel intervals down the column and any myelin contacting the line was quantified (up to 50 intact appearing and 50 degraded myelin areas per animal). Intact appearing myelin is defined as myelin that surrounds a degenerating axon. Degraded myelin is defined as myelin that is observed without an accompanying axon. Each individual axon and myelin sheath was manually circled to determine
myelin area; large watery spaces (areas of water trapped within the myelin sheaths, not between compacted bilayers) were highlighted using an intensity gradient (Adobe Photoshop, San Jose, CA). This approach required that image brightness levels be adjusted such that the large water spaces within the myelin sheaths were set to maximum intensity and myelin sheaths were set to zero intensity. The result was then thresholded to identify the watery spaces. The process is shown in Figure 5.2.

![Figure 5.2. Measuring large watery spaces in myelin debris.](image)

Figure 5.2. Measuring large watery spaces in myelin debris. Crop of a 10,000× TEM micrographs showing a single myelin debris is used to illustrate the measurement process. Selected myelin sheath (left) is manually segmented for myelin area (middle) and then thresholded using an intensity gradient to separate the large watery spaces from the compacted bilayers in the myelin area. Red scale bar is 1 μm.

### 5.2.2 Results and Discussion

Figure 5.3 shows the results from TEM analysis. The plot of water space area versus myelin area reveals two distinct populations: one consisting of intact appearing myelin and one of myelin debris. A significant difference ($p < 0.001$) was found in MWF between the 3 weeks group (0.589) versus the 8 weeks group (0.293), which corresponds well with previous results (47). Table 5.2 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.

![Graph showing the area of large watery spaces between intact appearing myelin and myelin debris.](image)

**Figure 5.3. Difference in the area of large watery spaces between intact appearing myelin and myelin debris.**

Area of large watery spaces within the myelin sheath is plotted against the manually segmented myelin and axon area. There the slope presents the ratio of watery spaces within the myelin and axon area. Myelin debris appeared to have more areas occupied by large watery spaces for a given myelin and axon area than intact appearing myelin. The ratio of large watery spaces to myelin and axon area was roughly three times higher in myelin debris.

**Table 5.2. MWF and T2 times for the short and long T2 component at 3 and 8 weeks post injury.** With the exception of MWF ($p < 0.001$), changes were not statistically significant.

<table>
<thead>
<tr>
<th>Time</th>
<th>3 Weeks</th>
<th></th>
<th>8 Weeks</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MWF</td>
<td>T2short</td>
<td>T2long</td>
<td>MWF</td>
</tr>
<tr>
<td>Mean (ms)</td>
<td>0.59</td>
<td>14</td>
<td>32</td>
<td>0.29</td>
</tr>
<tr>
<td>S.D. (ms)</td>
<td>0.08</td>
<td>2</td>
<td>6</td>
<td>0.03</td>
</tr>
</tbody>
</table>
The water space area versus myelin area results suggest that there are two to three times the amount of watery spaces in myelin debris than in intact appearing myelin, which should lead to a decrease in MWF measurement, as these large watery spaces within the myelin sheath, with spacing on the order of a few hundred nanometers, would be classified as I/E water.

Because of the challenges in obtaining consistent fixation quality of TEM data (see Chapter 3), the intact myelin vs myelin debris data was inconsistent and limited. While they tell us that the two are different in the amount of these large watery spaces, there was not enough data for a proper comparison of the injury time points or to resolve the actual myelin lipid bilayer spacing. However, we can speculate on how changes in bilayer spacing would affect some of the MR parameters: a small (few nm) increase in spacing should lead to larger myelin water component and longer myelin water T2. Webb *et al.* have previously demonstrated in the PNS that MWF measures both myelin and myelin debris (52). If the myelin sheaths were to loosen in myelin debris, in a mixed population, myelin debris would contribute to MWF more than intact myelin. Therefore, while MWF correlates well with myelin content in the absence of myelin debris, it may not be a good indicator of myelin content when there is a mixed population of intact myelin and myelin debris. This is especially critical in spinal cord injuries where there is a mix of intact myelin and myelin debris. We have seen in a previous study that in the presence of myelin debris, MWF can overestimate the amount of myelin content (47).

Common to histology studies is the question whether it truly reflects the situation *in vitro*, such as highlighted by the discovery and naming of the mesosome (122,123), which turned out to be a
fixation artifact. The large watery spaces shown here are unlikely fixation artifacts (124). The edge of the discontinuities in the myelin sheaths, where tissue integration is low, are well defined and unlike the blurred smears that would result from poor fixation. When the lamellae split, they follow the major or minor dense line around the sheath, which is unlikely to be the result of mechanical damage in the fixation process. All these suggests that the myelin debris is well fixed.

5.3 Conclusions

The first pilot study essentially replicated the results from previous studies with the addition of electron microscopy data. The focus on the fasciculus gracilis 5 mm cranial at 3 weeks post injury time point was motivated by the increased MWF seen in previous studies of complete DC Tx (47,74). Because of the nature of the injury, no intact myelin was expected; however, we did not find a significant difference in MWF between normal control and 3 weeks post injury. This suggests that MWF does not distinguish between myelin and myelin debris in the CNS, similar to the PNS results reported by Webb et. al. (52). The diffusion results showed significant reduction in FA, and $D_{\text{long}}$, indicating axonal degeneration (116,118). We also saw a significant increase in $D_{\text{trans}}$, as a result of both myelin and axonal degeneration, with the formation of large watery spaces within the degenerating myelin sheaths, seen on electron micrograph, being one of the possible contributor.

In the follow-up pilot study, we have shown that myelin debris has increased large water spaces within the sheath, compared to intact appearing myelin. It is likely that as the myelin sheath
degenerates, the area occupied by these water spaces increases. Because of their size, MWF would classify them as I/E water. Therefore, the still compacted portion of myelin debris needs to be studied further.

Overall, the results presented in this chapter suggest that MWF is not an accurate measure of the content of intact or damaged myelin when myelin debris is present. The TEM images are of high quality. As a result, it was used extensively in the next chapter to investigate whether MWF still reflects the total myelin content.
Myelin is essential for normal functioning of the nervous system by allowing efficient and rapid propagation of action potentials. Quantitative T2 based myelin water imaging (MWI) can measure myelin content in normal and diseased brain and spinal cord tissue (45,47). Direct imaging of myelin with MRI is difficult because the majority of the signal from protons associated with myelin has decayed by 3 ms (101). Therefore, quantitative T2 based MWI assesses myelin content by probing the properties of the water trapped between the myelin lipid bilayers (myelin water). Although some people argue that MWI constitutes a direct measurement method because the trapped water is an integral component of the myelin (32), such argument falls apart if changes in myelin morphology alter the relationship between myelin water and myelin content.

Typically, analysis of the T2 decay curves obtained with Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence yields three distinct T2 components in the brain and spinal cord tissues. The three components are attributed to water trapped between the myelin lipid bilayers, intra/extracellular water, and cerebrospinal fluid (77). Association of the short T2 component with myelin water is backed up by histological analysis showing good correlation between the myelin water fraction (calculated as the integral of the myelin water peak over the total integral of the T2 distribution) and myelin content, and has been successfully applied in studying the demyelination process in multiple sclerosis (53,54) and spinal cord injury (SCI) (47). In these studies, MWI were validated using optical microscopy, which has a resolution limit of approximately 0.2 micron. And while the use of immunohistological staining can provide certain molecular information, it
is not able to provide direct access to the fine, nanometer scale structure of myelin (ultrastructural scale), which directly impacts the relationship between MR based myelin water imaging technique and myelin, including quantitative T2.

Because the short T2 component is related to the signal from water trapped between myelin lipid bilayers, changes in the spacing between these bilayers can influence both the myelin water pool size and its T2 values. If these changes in the local tissue environment occur over time, it may make interpretation of the MWF difficult. This is especially important in acute spinal cord injuries, where large amounts of myelin debris are present throughout retrograde and Wallerian degeneration. Thus, one may expect a very different relationship between MWF and myelin content in myelin debris, vs. intact myelin, due to myelin morphological changes. To test whether ultrastructural differences between intact myelin and myelin debris indeed affect the relationship between MWF and myelin content, I compared myelin measurements derived with MRI and transmission electron microscopy (TEM) in a rat spinal cord injury model. More specifically, MWF was compared to TEM derived measurements of compact myelin content in both intact myelin and myelin debris.

A C5 dorsal column transection (DC Tx) provides clean injury model to study MWI in the presence of myelin debris. Based on previous findings using this model (47), I expect exclusively myelin debris at 3 weeks post-injury, which is partially cleared by 8 weeks post-injury in the fasciculus gracilis at 5 mm cranial/distal to the injury site (refer to Figure 1.13 for illustration). The fasciculus gracilis was chosen because it is an ascending tract with virtually no intermingling of neuronal cell bodies at the cervical level, which allows us to compare
contiguous sections. The use of separate sections enables each to be differently fixed in order to satisfy the conflicting requirements of high-resolution TEM and MR. It is also closer to the sample’s edge and therefore expected to be better fixed, especially in the injured cords where tissue integrity is reduced.

The term myelin debris is used here to describe non-normal-appearing myelin, including empty and/or fragmented/collapsed myelin sheath that still appears to retain their unique lipid bilayer structure.

6.1 Methods

6.1.1 Animal Preparation

All experimental procedures were carried out in compliance with the guidelines of the Canadian Council for Animal Care and approved by the local Animal Care Committee prior to conducting the study.

Eighteen male Sprague-Dawley rats (250–280 g) were randomly divided into three groups: injury group studied 3 weeks post-injury (six animals), injury group studied 8 weeks post-injury (six animals), and control group (six animals). The animals were housed in a standard rodent care environment with food and water freely available. DC Tx was induced at the C5 level in the post-injury groups as described in §1.3.4.1.

To excise the spinal cords, rats were deeply anesthetized and perfused intracardially with phosphate buffered saline (PBS) for 3 min, followed by freshly hydrolyzed paraformaldehyde.
(4%) and glutaraldehyde (1%) in 0.1M sodium phosphate buffer at pH 7.4. Spinal cords were then harvested and placed in the same fixative for 30 minutes on ice before being divided for TEM and MRI as shown in Figure 6.1.

![Figure 6.1. Division of spinal cord samples for MR and TEM.](image)

Figure 6.1. Division of spinal cord samples for MR and TEM. The 3 mm section length for MRI allows easier sample orientation in the sample holder. The MRI slice was 0.5 mm thick, and positioned as close as possible to the top edge of the sample, which coincided with the bottom edge of the sample used for TEM.

### 6.1.2 MRI Experiments

The 3 mm spinal cord sections were further postfixed overnight in 2% glutaraldehyde before scanning. All MRI experiments were carried out on a 7-Tesla preclinical scanner (Bruker BioSpin GmbH, Ettlingen, Germany) using a 13 mm inner-diameter and 25 mm long, five-turn, transmit/receive solenoid coil. Each of the excised cord section was transferred into a 4.5 mm inner diameter plastic tube filled with the fixative solution for scanning. Two plastic rods were used to prevent in-plane and vertical movements of the cord samples.
A single slice multi-echo CPMG sequence was used to acquire quantitative T2 data (21) with the following parameters: 1500 ms repetition time (TR), 6.738 ms echo time (TE) and echo spacing, 256 × 256 matrix size, 32 echoes, 1.79 cm field-of-view, 0.5 mm slice, and 12 averages, giving an in-plane resolution of 70 μm and scan time of 77 minutes. Slice location was prescribed as close to 5 mm cranial to injury as practical (Figure 6.1), ensuring that the MR and TEM slices are no more than a millimeter apart.

6.1.3 TEM Experiments

The 1 mm spinal cord sections were postfixed in 2% glutaraldehyde for protein fixation for 1 hour at 4°C. The sections were then washed in cacodylate buffer for 10 minutes (at 4°C) and placed in 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 M cacodylate buffer for 1 hour for lipid fixation (at 4°C). Afterward, the sections were rinsed in distilled water for 10 minutes before being stained with 2% uranyl acetate in ethanol for 30 minutes (4°C). Finally, the sections were dehydrated by washing them in successively higher concentrations of ethanol (2 × 5 minutes in 50%, 2 × 5 minutes in 70%, 2 × 5 minutes in 90%, 3 × 10 minutes in 95%, and 3 × 10 minutes in 100%) at 4°C. Before embedding, the sections were rinsed at room temperature in propylene oxide (3 × 20 minutes), infiltrated with 1:1 propylene oxide and Spurr’s resin (4 to 5 hours), infiltrated with 1:3 propylene oxide and Spurr’s resin (15 to 16 hours), and finally infiltrated with 100% Spurr’s resin for 5-6 hours. The resin was polymerized for at least 16 hours at 60°C in plastic molds until hardened into resin blocks.
Figure 6.2. Low magnification TEM images showing sample support grid. Top left shows the entire copper sample support grid under low magnification TEM. Bottom left shows a closer view of the *fasciculus gracilis*, with the border between resin and sample visible. The right image shows the central windows from the bottom left image at a higher magnification.
Figure 6.3. Sampling the *fasciculus gracilis* in TEM. Top left image shows the stitched optical image used as a road map. The right image shows an ideal, three windows sampling scheme. Bottom left shows the optical and TEM grid registered together, used to locate the area of interest. The red and green lines highlight the middle of the *fasciculus gracilis*.

From the resin blocks, 1 μm thick sections were made of the entire spinal cord cross section on a Leica Ultracut T (Leica Microsystems AG, Wetzlar, Germany) ultramicrotome using a 45° glass knife. The sections were stained with 0.5% toluidine blue and imaged at 20× magnification to serve as references for the TEM imaging. A Zeiss Axioplan 2 microscope (Carl Zeiss AG, Oberkochen, Germany) was used for these optical images, which were processed and stitched using ZEN Digital Imaging for Light Microscopy software (Carl Zeiss GmbH, Jena, Germany).

Using the same ultramicrotome, 70 nm thin sections were cut, mounted on copper SPI TEM grids (Figure 6.2), and counterstained with 2% uranyl acetate for 12 minutes, and with Reynolds’ lead citrate for 6 minutes. Three 80 μm × 80 μm grid windows in the fasciculus gracilis were chosen from each section (Figure 6.3). Within each window, 9 TEM images were taken in a 3 ×
3 pattern, spaced 10 μm apart, at 16,000× (5.83 μm × 5.83 μm at 2048 × 2048) and then again at 35,000× magnification (1.3 μm × 1.3 μm at 2048 × 2048). A total of 54 images per spinal cord section were acquired at a resolution of 2048 × 2048 and 16-bit grayscale depth. All TEM images were generated on a Hitachi H7600 transmission electron microscope (Hitachi Ltd., Tokyo Japan) using a built-in AMT digital camera (American Technologies Corp., San Francisco, CA) and iTEM analysis software (Olympus Soft Imaging Solutions GmbH, Münster, Germany, version 5.0).

6.1.4 Data Processing and Analysis

CPMG data was processed using in-house developed Matlab (Natick, MA) code that performs regularized non-negative least square analysis (18,77). T2 decay curve from each voxel was fitted individually to 101 logarithmically spaced T2 times from TE (6.738 ms) to TE+TR (1506.739 ms) by minimizing both $\chi^2$ and an energy constraint. The energy constraint was set to 2-2.5% above the minimum $\chi^2$ to generate a smoothed T2 distribution, which results in a more consistent fit in the presence of noise, than in the case of non-regularized fit (58,59).

Myelin water fraction (MWF) maps were generated by dividing the sum of the amplitudes from 6.738–13 ms range by the total sum of amplitudes of the T2 distribution for each voxel. The use of 13 ms instead 20 ms as the upper cutoff for MWF calculation was due to the shortened T2 caused by the switch from 4% paraformaldehyde fixative to the 2% glutaraldehyde fixative (see §4.2 for details). Examples of T2 distributions from each group are shown in Figure 6.5. Regions of interest in the fasciculus gracilis were defined as every pixel that touches a line drawn midline.
of the *gracile* (Figure 6.4). Region of interest analysis was then used to obtain the average MWF from every voxel that touches this line (121).

**Figure 6.4. ROI sampling scheme for MWF map.** ROI was defined as any voxel touching the midline of the *fasciculus gracilis*. Left shows the first echo image; right shows the MWF map.

<table>
<thead>
<tr>
<th>T2 (ms)</th>
<th>MWF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>MWF = 0.38</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
</tr>
<tr>
<td></td>
<td>MWF = 0.34</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
</tr>
<tr>
<td></td>
<td>MWF = 0.25</td>
</tr>
</tbody>
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**Figure 6.5. Sample T2 distributions.** Example T2 distributions and their respective MWF values for the three datasets are shown here. The cut-off for MWF calculation is set at 13 ms. The T2 spectrum is only shown up to 200 ms and the rest are truncated because the fit produced zero amplitude.
TEM images at 16,000× magnification were used to quantify the myelin content. The full 16-bit grayscale was normalized to be between 0 and 1 by mapping the 0.1 to 99.9 percentile pixels linearly. These images were then processed using an 8 × 8 adaptive Wiener filter (125) to reduce image noise, followed by a contrast-limited adaptive histogram equalization (126) (8 × 8 tiles and 256 bins) to correct for uneven illumination, and to produce a flat histogram. Myelin area was manually segmented and thresholded to exclude large watery spaces (vacuolated formations) that formed between separated or discontinuous myelin lamellae (Figure 6.6). The resulting area was then divided by the total area to determine the myelin fraction. The ratio of large watery spaces to myelin area was also calculated. The TEM produced myelin fraction, and myelin area (including the large watery spaces) was then correlated to the MR-derived MWF. Bland-Altman test was performed to test for a potential bias and to analyze the agreement between two different methods. It is a plot of the difference versus the mean of the two methods calculated for each animal.

TEM images at 35,000× magnification were used to determine myelin periodicity. Myelin spacing/period was sampled once in each image, from a randomly selected myelin sheath, by measuring and counting the major dense lines, as shown in Figure 6.7. Differences in myelin spacing, MWF, myelin fraction, and ratio of large watery spaces to myelin fraction among the three groups (normal, 3 weeks post-injury, and 8 weeks post-injury) were compared using one-way ANOVA. The significance of the results was tested using a post-hoc Tukey test.
Figure 6.6. **TEM processing pipeline.** From the TEM image (left), myelin area was manually segmented (middle) and thresholded (right) to determine myelin fraction. Myelin fraction was calculated as the area fraction in black. The red arrow points to an especially darkly-stained piece of myelin.

Figure 6.7. **Example of myelin period measurement.** Myelin period was sampled once in each image from a randomly selected myelin sheath by measuring and counting the major dense lines. The right image shows the 100% crop area highlighted on the left image in red. Whenever the myelin lipid bilayers are resolved clearly it is implied that they are oriented perpendicularly within the section; otherwise, they would be blurred due to the difference in the size of the lipid bilayer and the section thickness (~11 nm versus ~70 nm). The blue line is 225 nm long.
6.2 Results

In total, four samples were lost due to inadequate TEM fixation, leaving us with three normal control, five 3 weeks post-injury, and six 8 weeks post-injury pairs.

Results of MWF versus myelin area comparison are shown in Figure 6.9. There is a trend of increasing MWF versus the manually segmented myelin area with the watery spaces included ($R = 0.47$, $p = 0.087$). After the removal of watery spaces, I found a very strong correlation between MWF and myelin fraction ($R = 0.82$, $p < 0.001$), as shown in Figure 6.9. The Bland-Altman results revealed no systematic bias. No significant differences were found in the myelin spacing between normal ($M = 10.9 \text{ nm}, SD = 3.0 \text{ nm}$), 3 weeks, ($M = 11.2 \text{ nm}, SD = 1.9 \text{ nm}$), and 8 weeks ($M = 11.0 \text{ nm}, SD = 2.6 \text{ nm}$) post-injury cords, as shown in Figure 6.10 ($F(2,378) = 0.80$, $p = 0.45$). ANOVA performed on MWF values indicated that all experimental groups differ significantly from one another ($F(2,18) = 37.68$, $p < 0.001$, see Figure 6.11). The MWF was $0.43 \pm 0.01$ for normal control, $0.34 \pm 0.02$ for 3 weeks, and $0.26 \pm 0.05$ for 8 weeks post-injury. Post hoc Tukey test yields $p = 0.033$, $0.026$, and $0.0012$ for 3 weeks versus 8 weeks, 3 weeks versus normal, and 8 weeks versus normal respectively. ANOVA performed on myelin fraction yielded $F(2,13) = 23.81$ and $p < 0.001$, as shown in Figure 6.12. Post hoc Tukey test yield significant differences in mean of 8 weeks post-injury group versus the other two groups ($p = 0.001$, $0.27$, and $< 0.001$ for 3 weeks versus 8 weeks, 3 weeks versus normal, and 8 weeks versus normal respectively). ANOVA perform on the water space to myelin fraction ratio yielded significant variations, $F(2,378) = 22.87$, $p < 0.001$. A post hoc Tukey tests show that the 3 weeks ($0.71 \pm 0.51$) and 8 weeks ($0.83 \pm 0.68$) post-injury groups differed significantly from the
normal control (0.25 ± 0.16) at $p < 0.05$; the two injured groups were not significantly different.

A plot of the watery spaces to myelin fraction ratio is shown in Figure 6.13.
Figure 6.8. MWF versus myelin area. MWF is plotted against the manually segmented myelin area (myelin fraction + large watery spaces). The correlation between the two measures is moderate but not significant ($N = 14$, $R = 0.47$, $p = 0.087$). The least-square regression line is shown.

Figure 6.9. MWF versus myelin fraction. The left graph shows the best-fit line and 95% confidence interval. The right graph shows the result of Bland-Altman analysis. The two measures are very strongly correlated ($N = 14$, $R = 0.823$, $p < 0.001$), the (0,0) point lies within the confidence interval, and there does not seem to be a systematic bias. There is very little change in the correlation coefficient value if the intercept is forced to zero ($R = 0.816$).
Figure 6.10. Comparison of myelin period between normal, 3, and 8 weeks. No significant differences ($F(2,399) = 0.80, p = 0.45$) were found in the myelin spacing between normal ($M = 10.9 \text{ nm}, SD = 3.0 \text{ nm}$), 3 weeks ($M = 11.2 \text{ nm}, SD = 1.9 \text{ nm}$), and 8 weeks ($M = 11.0 \text{ nm}, SD = 2.6 \text{ nm}$) post-injury. The box plot shows the sample median. The top and bottom of the box indicate the 25$^{th}$ and 75$^{th}$ percentile. The whiskers are set at 1.5 times the interquartile range. Notches in the box show the variability of the median and are computed such that non-overlapping notches have different medians at the 5% significance level. Outliers are denoted by +.
Figure 6.11. Comparison of MWF between normal, 3, and 8 weeks. The scatter plot shows the MWF from each of the three time points. All groups differ significantly from one another ($F(2,18) = 37.68, p < 0.001$). The MWF was $0.43 \pm 0.01$ for normal control, $0.34 \pm 0.02$ for 3 weeks, and $0.26 \pm 0.05$ for 8 weeks post-injury.

Figure 6.12. Comparison of myelin fraction between normal, 3, and 8 weeks. The scatter plot shows the myelin content from each of the three time points. The myelin fraction was $0.41 \pm 0.01$ for normal control, $0.38 \pm 0.05$ for 3 weeks, and $0.24 \pm 0.03$ for 8 weeks post-injury. Only the 8 weeks post-injury group is significantly different from the other two groups.
Figure 6.13. **Large watery spaces as a portion of myelin fraction.** The ratio of large watery spaces to myelin fraction is shown as a function of myelin fraction to illustrate the changes in large water spaces as myelin degenerates into myelin debris and the subsequent clearing of this debris. Each point corresponds to one TEM image.

### 6.3 Discussion

The TEM myelin fraction correlated strongly with MWF and significant differences were found in myelin content among the experimental groups. TEM images suggest that as intact myelin degenerates into myelin debris, there were little ultrastructural changes (changes in biological structures smaller than observable by optical microscopy, but larger than molecular). The exception was for the formation of large watery spaces between myelin discontinuities or
separated lamellae (Figure 6.14 and Figure 6.15). The delamination was likely due to loss of protein from the myelin (127).

The MWF reported here are higher than previously measured in a similar study *in vivo* (84). This is likely due to the TEM fixation requirement. In order to resolve lipid bilayers, the use of glutaraldehyde, in addition to PF, results in higher cell shrinkage due to water removal predominantly from the intra/extracellular space (128). The preferential removal of intra/extracellular water explains the consistently higher MWF values in fixed cords than *in vivo*, as observed in previous studies (84). Overall the periodicity measured in this study matches the literature value for osmium fixed CNS myelin periodicity of between 10.5 and 11.5 nm (32–34), including isolated globular masses. Also reassuring is the fact that wide angle x-ray diffraction experiments of unfixed tissue confirm the same spacing, demonstrating that fixation does not affect the periodicity (129).

The manually segmented myelin area already trends towards a positive correlation with MWF, even before the removal of large watery spaces, as shown in Figure 6.8. This is unsurprising because the amount of large watery spaces depends on the amount of myelin. However, the correlation becomes highly significant after the removal of these large watery spaces, mainly due to the significant increase in these spaces in myelin debris, when compared to intact myelin. The results suggest that MWF accurately reflects the amount of myelin lipid bilayers, regardless of the state of the myelin. This also explains why Luxol Fast Blue correlates so well with MWF in earlier studies (47,54,105): because Luxol Fast Blue is a lipophilic dye that stains lipoprotein,
both intact myelin and myelin debris that contain myelin lipoproteins that have not been phagocytosed would have been stained indiscriminately (130).

Throughout Wallerian degeneration, collapsed/peeled myelin appears to consist only of areas of either approximately normally spaced myelin (with little ultrastructural changes) or large spaces (see Figure 6.14 and Figure 6.15) formed in between separated or discontinuous lamellae. The lack of staining in these spaces suggests that these spaces are high in water content, although amorphous lipid was likely present (131). One proposed mechanism is that, as areas in myelin sheaths break down, they disintegrate into smaller and smaller molecules which increase the osmotic pressure and draws in water, creating large watery spaces (124). However, it should be noted that these spaces are also observed in normal control in other studies as well (124,132–134). Nevertheless, it seems that whatever molecular or supramolecular changes that must have taken place during the process of intact myelin becoming myelin debris, they do not affect the MWF, and that as long as the myelin lamellae are visible, they retain their characteristic bilaminar ultrastructure. Therefore, the area of normally spaced bilayer in myelin debris would contain a similar amount of water as normal healthy myelin, while the large watery spaces would be classified as intra/extracellular water by quantitative T2 analysis.

Figure 6.13 provides another look at this process. Since each point reflects a single TEM image containing no more than a few myelin sheaths, the graph captures the details at this scale. In the normal control samples, there is a tight clustering in both the myelin fraction and ratio of watery spaces. This is expected, as the healthy tract should appear homogeneous. At the 3 weeks post-injury, there is an increase in the amount of watery spaces and an increase in the spread of
myelin fraction after complete degeneration of the *fasciculus gracilis*. The disappearance of axons allows for both area of high myelin fraction (as the myelin sheaths collapse into spaces that would otherwise be occupied by the axons without forming additional watery spaces), and areas of low myelin fraction (as the myelin sheaths expand by forming large watery spaces to fill the area that would otherwise be occupied by the axons). These can be seen in Figure 6.14 and Figure 6.15. At 8 weeks post-injury, the myelin fraction is reduced as the debris are partially cleared, and the spread is even more exaggerated with lower myelin fraction overall and increased space for the debris to expand into. The significant difference in the amount of large watery spaces between intact myelin in the normal control group and the myelin debris in the injured groups confirms the pilot data presented in §5.2.2.

Curiously, while the results here demonstrate no significant difference in myelin spacing, *in vivo* data in the same WM track using the same rat model have shown higher MWF reading at 3 weeks post-injury than normal control (84). This may suggest that factors other than morphology of myelin debris need to be considered when imaging *in vivo*. One source could be the inter-compartmental water exchange between myelin and non-myelin tissue. The collapse of the myelin into areas previously occupied by the axons and the formation of large watery spaces could mimic the microstructure of thickly myelinated axons, which can inflate the MWF reading, when exchange plays a large role, such as in *in vivo* situations (93,110). The lower temperature of the *ex vivo* experiment could see a much-reduced contribution from water exchange. In general, water exchange causes underestimation of MWF (110). If water exchange between the myelin water and the intra/extracellular component becomes much slower due to the reduction in temperature from high 30°C to low 24°C, then the measured MWF at the lowered temperature
will be both larger and closer to the true value (76). Compared to previous in vivo studies that showed elevated MWF measurement at 3 weeks, water exchange may be less of a factor here because scanning in 2% glutaraldehyde does not significantly impact exchange rate (102), unlike with paraformaldehyde.
Figure 6.14. Example of myelin debris. The spacing remains fairly consistent throughout most of the myelin debris. Only at the edge of the large watery spaces and sharp turns some loosening of myelin bilayers is evident. In this instance, the myelin sheath collapsed inward without forming many large watery spaces.
Figure 6.15. Another example of myelin debris. The myelin sheaths on the left and right expanded into spaces previously occupied by the axon by forming large watery spaces. Outside of the watery spaces the myelin retains its normal, compact spacing.
For the MWF to represent the actual myelin fraction, one would expect the MWF to be zero when no myelin is present. The linear fit of the MWF vs. myelin fraction in this study has a zero crossing at MWF = 0.075. This is most likely due to a lack of data points with myelin fraction close to zero in the results, as (0,0) lies within the 95% confidence interval. In fact, when the fit is forced to cross zero at MWF = 0, the correlation remains nearly as strong with a correlation coefficient that is only marginally lower (0.816 vs. 0.823).

The slope of the correlation graph is 0.75, which becomes 0.96 if the intersect is forced through zero. The Bland-Altman analysis suggests that these methods are interchangeable, but this creates a fundamental problem. The myelin fraction I used to measure myelin content is the area fraction occupied by myelin with compact membrane layering, while MWF is associated with the water trapped between these layers. Given that myelin contains approximately 40% water and intra/extracellular compartment contains approximately 70% to 90% water (135,136), the relationship between MWF and myelin fraction (MF) can be calculated as

$$MWF = \frac{MF \cdot 40\%}{MF \cdot 40\% + (1 - MF) \cdot \%Water_{\text{IE}}} ,$$

which is not linear.

Consequently, in this simplistic model, the MWF readings shown in the results are too high, even when an additional 10% shrinkage to intra/extracellular space was added, as demonstrated in Figure 6.16. That said, tissue shrinkage of 40% to the intra/extracellular space would explain the discrepancy; however, this would introduce a rather large change in the sample’s volume, which, from casual observations, did not appear to be the case. A whole sample water content
measurement might provide a better insight. Also, the inter-compartmental water exchange could play a role, as the lower exchange rate tends to increase the apparent MWF, as could the effect of chemical fixation. In addition, the correspondence between the ex vivo MR data and the TEM data is not fully understood (110). Ultimately, there are many possible processes at work, which warrant further investigation.

![Graph](image)

**Figure 6.16. Expected MWF versus myelin fraction.** MWF calculated using Eq. (6.1) is plotted against the myelin fraction. The three lines show the expected MWF for a given myelin content at various intra/extracellular water contents. Myelin water content is assumed to be 40%. The 63% line shows the result of a further 10% shrinkage in intra/extracellular space from 70% due to fixation.

The strong correlation between MWF and myelin content, in the case of both functioning myelin and myelin debris, poses a limitation on the MWI technique, because MWF is unable to
distinguish between the two. Perhaps a multi-parametric approach, that combines two or more
MRI methods, can provide more insight into the demyelination process. For example, if a
diffusion-based MRI technique indicates axonal degeneration without any change in MWF, one
may conclude that MWF, in this case, represents mostly myelin debris with compact membrane
layering. Unfortunately, basic parameters derived from DTI are influenced by a combination of
factors, such as the longitudinal diffusivity being dependent on both myelin integrity and intra-
axonal composition (137). In any case, the approach suffers from lengthy scanning time.

6.4 Conclusions

The results of this study demonstrate that MWF correlates strongly with the amount of myelin
lipid bilayers in both intact myelin and myelin debris. As myelin degenerates, it tends to form
large watery spaces within the myelin sheaths that are classified as intra/extracellular water in
MWI. The lipid bilayer spacing otherwise remains as that of normal intact myelin. Despite its
inability to distinguish between intact myelin and myelin debris that is still compact, MWI is a
useful tool for assessing actual myelin content even in the presence of debris.
Chapter 7: Conclusions

The two goals of the research presented in this thesis were 1) to validate the use of myelin water imaging (MWI) in the presence of myelin debris and 2) to optimize MWI by utilizing compressed sensing (CS) for improved acquisition speed.

Overall, I demonstrated that the correlation between myelin water fraction (MWF) and myelin content is retained in both intact myelin and myelin debris, because, as intact myelin degenerates into myelin debris, little ultrastructural changes occur, except for the formation of large watery spaces. My results enhanced the understanding and interpretation of MWF in the presence of myelin debris. I incorporated CS into MWI and showed that it improved acquisition efficiency in both 2D and 3D, and that the sampling requirements are different between the two cases.

7.1 Compressed Sensing

While we have had great success in *ex vivo* studies of MWI in rat spinal cord using multi-echo CPMG sequence, this technique is inherently slow, especially for high-resolution *in vivo* animal studies, where there is a conflicting requirement of short scanning time and high signal-to-noise ratio (SNR). We hypothesized that using CS with group-sparse reconstruction will significantly increase the acquisition efficiency of MWI. CS accelerated MWF maps were simulated using fully sampled $k$-space data acquired from excised rat spinal cords at 7T. We found that MWF map quality was minimally impacted up to an acceleration factor of two, making the technique a promising approach for increasing acquisition efficiency in MWI. Although in our *in vivo* studies of rat spinal cord the SNR is typically at the threshold for accurate MWF quantitation, which
makes the application of CS CPMG difficult, the CS technique developed here can be easily applied to other \textit{in vivo} studies, where SNR is less of an issue, \textit{e.g.} human brain imaging or preclinical studies with the use of cryprobe.

The 2D CS study was, naturally, extended into 3D, where I found the same improvement. Furthermore, I later discovered in simulation CS experiments that my 3D acquisition could be improved by using an identical sampling scheme for all echoes. The work done here contributes to the expanding use of CS in MR in the last decade as the technique moves beyond spatial domains. Some readers may have noticed that CS was not used in the subsequent parts of the thesis after the technique was present. This is because chronologically, the investigation into the use of CS for MWI, despite being presented first, actually came after the investigation into MWI in myelin debris.

### 7.2 Myelin Debris

The project started when we observed high MWF values in a rat model after dorsal column transection injury. The complete interruption of the dorsal column means that for the extent of the axon from the injury site, there should not be any functional axons or myelin sheaths left. So we were curious, how does MWI behave in such situation?

Quantitative T2 based MWI measures myelin content by probing the properties of the water trapped in myelin and therefore depends on its morphology. I compared MR MWF to transmission electron microscopy (TEM) derived myelin content using a rat injury model, and found that MWF correlates strongly with the amount of myelin lipid bilayers in both intact
myelin and myelin debris, and that myelin debris appears to consist of areas of either normally spaced myelin or large watery spaces. No significant differences were found in myelin spacing among normal, 3 weeks, and 8 weeks post-injury time points. Part of the novelty of this project comes from the use of high-resolution TEM, which had faded since the 1960’s, as researchers moved on to other modern histological techniques with higher specificity that are less labour intensive (fluoroscopy, immunohistology, etc.). Few works in spinal cord injury had been done at such a high resolution since then, and rarely in conjunction with MR.

The project was not without hurdles. We had difficulty achieving well-fixed spinal cord samples for high-resolution TEM while simultaneously satisfying the MR imaging requirements. In our attempts to improve the quality of the fixation, we observed a new phenomenon that creates a loss of MWF map contrast shortly after perfusion fixation. Eventually, we settled on using contiguous slices for MR and TEM instead of a single slice for both. Along the way, we tested whether buffer concentration had any effect on MWF because we were curious about the role of osmolarity in our studies. It did, but was easily corrected for.

7.3 Future Work
A drawback of the work presented in this thesis is that all MR scans were done with the sample immersed in fixative, which shortens the T2 to varying degrees depending on the fixative used. This is holdover from when the same samples needed to be kept well fixed for high-resolution TEM work. However, once it was decided that the samples were to be divided and processed separately for TEM and MR, there was no longer the need for this practice. For future
experiments, I would suggest washing the sample in buffered saline before performing MR experiments in order to return the T2 times to closer to physiological.

The work presented in Chapter 3 deserves further investigation. While the data showed convincing loss of the gray/white matter contrast in MWF shortly after perfusion fixation, it is limited and warrants a follow-up study with improved experimental design. This would likely be done with two groups of six animals, with one group consisting of perfusion fixed spinal cord samples and another of immersion fixed spinal cord samples. The perfusion fixed group will serve as the control group and the immersion fixation will be done inside the magnet. A large volume of fixative needs to be circulated through the sample holder to achieve proper fixation to ensure a proper time course of T2 and MWF changes could be studied. This is probably the easiest and most significant follow-up to the thesis. The same experimental setup will also give us fine temperature control over the sample. By coupling this with a thermometer, we can also investigate the effect of temperature on the MWF. Finally, we should also keep track of the volume of the sample to determine the amount of tissue shrinkage over time. Interestingly, in vitro experiments involving rat spinal cord are possible, and may be worth pursuing (138–140).

One of the methods discussed in Chapter 1 that was not used in the pilot study presented in §5.1 is magnetization transfer (MT). As mention in §1.5.1, MT lacks specificity for myelin because it is influence by many other factors. However, by using quantitative models and/or multi offset frequency/RF saturation pulses, more specific tissue parameters could be extracted. Of special note is the work done by Smith et al where quantitative MT derived parameters has been shown to be able to characterize abnormal myelin in the spinal cord (120). The sensitivity of
quantitative MT to tissue microstructure may offer a method to distinguish between intact myelin and myelin debris, thus warranting its inclusion in future follow-up studies.

Regarding the numerous large watery spaces that were the focus of Chapter 6, there is some debate as to how much of it is an artifact resulting from the fixation process. The choice of fixative for perfusion may also affect the appearance of TEM samples. It is likely that only small portions of these spaces are fixation artifacts. This can be independently verified with other methods, such as freeze fracture. This validation step is likely the most difficult follow-up to the thesis.

Several things from the CS portion remain to be done. It would be desirable to test 3D CS CPMG in vivo; however, it would require a setup that improves on our current best possible signal-to-noise ratio that also overcomes the geometric limitation of non-selective slice refocusing pulse (e.g. through coil design). The comparison between differently sampled and identically sampled echoes for 3D CS CPMG was done on a limited number of simulated undersampling datasets. More testing is needed, including actual undersampling experiments to strengthen this claim. Due to the nature of the CPMG sequence, identical echo sampling may confer an even bigger advantage over random echo sampling in actual CS experiments, as each echo is not individually phase encoded.

While CS improves the acquisition speed, CS image reconstruction is computationally expensive and suffers from long image reconstruction time. At the start of the project, a 32-bit operating system running Matlab 2007b was used. Even with most of the code compiled, on an Intel
Q9550 powered machine with 4GB of RAM, reconstruction would be an overnight affair. Moving to the 3D case, with sixteen times the amount of data (sixteen slices), the memory requirement necessitated 64-bit operating system, but even then, the reconstruction would take up a good part of the week. Over the years, through hardware and software upgrades and steady optimization of the reconstruction code, we were able to bring reconstruction time down to an hour (Windows 10, Intel i7 6700, 32 GB of RAM, Matlab 2016a) for the same dataset. Yet, there is more speed to be had. For example, porting the code from Matlab to a systems language not only opens up additional performance potential but also avoids Matlab’s closed proprietary system; and even within Matlab itself, there have been continual performance enhancements with each release. Further improvements to the reconstruction algorithm are also possible.

Finally, the osmolarity project needs some discussion. The MR portion of the results is exciting and carries with it important implications for any MR experiments that make use of phosphate buffer (PB). Based on the data presented here, many possible follow-up experiments can be designed. The first is to remove the contribution of the fixative itself, by flushing the samples in PB buffered saline over a period of time. This isolates the effect of PB. Simulations incorporating the effect of PB concentration on inter-compartmental water exchange can be designed and checked against MR data. It would be interesting to assess how the proton exchange between the different water compartments is altered by PB concentration, and how the associated MR parameters are affected. The use of PB at varying concentrations can provide a convenient and novel method for investigating the much-debated role of water exchange in myelin water imaging.
In comparison, the TEM portion of the study was less exciting, so in the interest of time and due to the sudden departure of a collaborator, we processed only a limited subset of data in order to focus on MWI in myelin debris. In fact, only nine out of twenty-four of the embedded samples were processed and imaged under an electron microscope. Because the samples do not degrade after embedding, should anyone choose to pursue the TEM portion of this project to completion, or simply wants to use it as practice on their way to mastering the ins and outs of electron microscopy, there exist twenty-four resin-embedded samples sitting in a drawer at ICORD.
References


120. Smith SA, Golay X, Fatemi A, Mahmood A, Raymond GV, Moser HW, van Zijl PCM, Stanisz GJ. Quantitative magnetization transfer characteristics of the human cervical spinal cord


Appendix

Osmium Fixed Sample in MR

Following the gray matter oddity presented in Chapter 3, it was decided to test whether any useful MR data can be acquired after the sample had undergone the lipid fixation stage with osmium tetroxide and potassium ferrocyanide (OsFeCN).

One would not expect such experiment to work, because the osmium in osmium tetroxide is a transitional metal that is paramagnetic, and the ferrocyanide in potassium ferrocyanide is diamagnetic. Nevertheless, since the experiments were done, the results are shown here in the appendix as a curious aside.

One normal spinal cord sample section was fixed following the protocol outlined in §6.1.3 up to and including the OsFeCN stage. The 8 mm cord section centered on C5 was divided into 1 mm slices and fixed individually. All experiments were performed on a 7 T animal scanner (Bruker, Germany) using a single-slice multi-echo CPMG sequence and a 13 mm inner-diameter solenoid coil for both spin excitation and signal reception. Quantitative T2 data (21) were acquired with the following parameters: 1500 ms repetition time (TR), 6.738 ms echo time (TE), 256 × 256 matrix size, 32 equally spaced echoes at 6.738 ms apart, 1.79 cm field-of-view, 0.5 mm slice, and 12 averages. NNLS fitting was performed using in-house developed Matlab (Natick, MA) code.

Figure A.1 shows examples of MWF map and representative T2 distributions obtained from these samples. NNLS SNR ranges from ~150 to ~180. Compared to PF/GD fixed cords, these
samples necessitate moving the cut-off of MW to 40 ms. ROI analyses of the *fasciculus gracilis* yielded an average MWF of 0.45 from the three 3 weeks post-injury samples and 0.45 from the single normal control sample.

**Figure A.1. Example MWF map and T2 distribution of OsFeCN fixed samples.** The MWF maps were often noisy despite the high NNLS$_{SNR}$. Middle and right columns show example WM and GM T2 distributions. Four of the eight samples from the normal cord are shown. Despite the erratic distributions, the GM/WM contrast persisted through OsFeCN fixation.

Curiously, several of the MWF maps showed a high MWF border around the sample, maybe due to OsFeCN binding to the other surface of the sample. And while the MWF map appears reasonable, the underlying T2 distributions were highly variable. Outside of the typical two-component distribution and poorly defined single peak distribution, voxels in WM often give rise
to 3 peaks (Figure A.1, middle, top two distributions). We speculate that this may be due to OsFeCN modifying membrane permeability (sealing the membrane and reduce exchange), or that OsFeCN not penetrating the plasma membrane well (which creates a unique extracellular pool). For now, this will have to remain just a curious aside.